#29

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE: U.S. PATENT NO. 5,914,331

ISSUED: JUNE 22, 1999

TO: EMORY UNIVERSITY

INVENTORS: DENNIS C. LIOTTA, RAYMOND F. SCHINAZI AND WOO-BAEG CHOI

FOR: ANTIVIRAL ACTIVITY AND RESOLUTION OF 2-HYDROXYMETHYL-5-(5-FLUOROCYTOSIN-1-YL)-1,3-OXATHIOLANE

MS Patent Extension
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

APPLICATION FOR THE EXTENSION OF THE TERM OF THE UNITED STATES PATENT NO. 5,914,331 UNDER 35 U.S.C. § 156

Applicant, Emory University of Atlanta, Georgia, represents that it owns the entire right, title and interest in and to Letters Patent of the United States No. 5,914,331, by virtue of assignments recorded in the United States Patent and Trademark Office on April 20, 1992 at Reel 6128, Frames 0085, 0089 and 0093.

Emory University has granted Power of Attorney to its licensee Gilead Sciences, Inc. to act as Attorneys for Applicant (EXHIBIT A).

Pursuant to the provisions of 35 U.S.C. § 156, Applicant hereby applies for an extension of the term of said United States Patent of 642 days, based on the materials set

forth herein and in the accompanying EXHIBITS A-H. In the materials that follow, paragraph numbers correspond to the paragraph numbers in 37 C.F.R. § 1.740(a).

(1) The approved product is EMTRIVA™ (emtricitabine) which is identified further as follows:

Chemical name:

5-fluoro-1-(2R,5S)-[2-(hydroxymethyl)-[1,3]-oxathiolan-5-yl]cytosine

Generic name:

emtricitabine

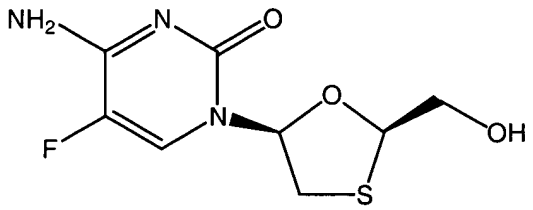
Molecular formula:

$C_8H_{10}FN_3O_3S$

Molecular weight:

247.24 gm/mole

Structural formula:



Physical form:

EMTRIVA is a white to off-white powder in capsules for oral administration. Each capsule contains 200 mg of emtricitabine and the inactive ingredients: crospovidone, magnesium stearate, microcrystalline cellulose and povidone.

(2) EMTRIVA (emtricitabine) was subject to regulatory review under subsection 505(b) of the Federal Food, Drug and Cosmetic Act (21 U.S.C. § 355(b)).

(3) EMTRIVA (emtricitabine) received permission for commercial marketing or use under subsection 505(b) of the Federal Food, Drug and Cosmetic Act (21 U.S.C. § 355(b)) on July 2, 2003 (See approved package insert, EXHIBIT B).

(4) The active ingredient in EMTRIVA is emtricitabine. Emtricitabine has not been previously approved for commercial marketing or use under the Federal Food, Drug and Cosmetic Act.

(5) This application is being submitted within the sixty-day period permitted for its submission pursuant to 37 C.F.R. § 1.720(f). The sixty-day period ends on Saturday, August 30, 2003. The last day on which this application could be submitted is Tuesday, September 2, 2003, which is the first business day thereafter.

(6) The patent for which an extension is being sought is identified as follows:

Assignee:	Emory University
Inventors:	Dennis C. Liotta Raymond F. Schinazi Woo-Baeg Choi
Patent No.:	5,914,331
Title:	ANTIVIRAL ACTIVITY AND RESOLUTION OF 2-HYDROXYMETHYL-5-(5-FLUOROCYTOSIN-1-YL)-1,3-OXATHIOLANE
Issued:	June 22, 1999
Expires:	September 29, 2015

(7) A copy of United States Patent No. 5,914,331, the patent for which an extension is sought, is attached hereto as EXHIBIT C.

(8) During the course of prosecution, U.S. Ser. No. 08/488,097, now US Patent No. 5,914,331, was terminally disclaimed over: (i) U.S. Ser. No. 08/402,730, pending; (ii) U.S. Ser. No. 08/482,875, now US 6,114,343; (iii) U.S. Ser. No. 08/474,406, now US 6,069,252; and (iv) U.S. Ser. No. 017,820, now US 5,814,639. Copies of the Terminal Disclaimers are attached hereto as EXHIBIT D.

A certificate of correction issued on March 11, 2003 in United States Patent No. 5,914,331. A copy of the certificate of correction is attached hereto as EXHIBIT E.

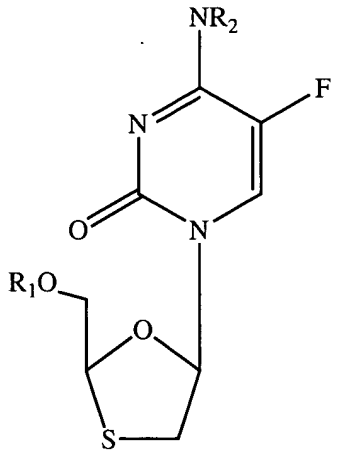
No reexamination certificate has issued in United States Patent No. 5,914,331.

The maintenance fee payment for year 4, the sole maintenance fee due, has been paid and the receipt received. A copy of the maintenance fee payment receipt is attached hereto as EXHIBIT F.

(9) United States Patent No. 5,914,331 claims the approved product. United States Patent No. 5,914,331 has seven (7) claims which read upon the approved product, its salts and esters and pharmaceutical compositions thereof.

(i) The manner in which at least one patent claim reads on the approved product is as follows:

Claim 1 (corrected) in its entirety is: A derivative of β-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxothiolane of the formula:



wherein R_1 is selected from the group consisting of hydrogen, acetyl, propionyl, butyryl, and pentanoyl and R_2 is hydrogen.

Emtricitabine is a compound of claim 1 wherein R_1 is hydrogen and R_2 is hydrogen. Therefore, claim 1 claims the approved product.

(10) (i) The relevant dates and information pursuant to 35 U.S.C. § 156(g) in order to enable the Secretary of Health and Human Services to determine the applicable regulatory review period for US Patent No. 5,914,331 as a patent claiming a human drug product are as follows:

(A) The effective date of the investigational new drug (IND) application was September 20, 1997 and the IND number was 53971.

(B) The date on which a new drug application (NDA) was initially submitted was September 3, 2002 and the NDA number was 21-500

(C) The date on which the NDA was approved was July 2, 2003.

(11) A brief description of the significant activities undertaken by the marketing Applicant, Gilead Sciences, during the applicable regulatory review period with respect to the approved product, emtricitabine capsules, and the significant dates applicable to such activities is attached hereto as EXHIBIT G.

The description includes interactions between the FDA, Gilead Sciences, Inc., and Triangle Pharmaceuticals, Inc., which entity originally submitted IND 53,971 and NDA 21-500. Gilead Sciences, Inc, acquired Triangle in January 2003 and continued development.

There was an agency relationship between the patent owner, Emory University, (the applicant for this extension) and the marketing applicants, Triangle and Gilead, during the entire regulatory review period as evidenced by the grant of a patent license to the marketing applicant predating the regulatory review period. Further, Gilead Sciences, Inc. has authorized Emory University to rely upon its activities before the FDA in seeking this extension (EXHIBIT H).

(12) Applicants are of the opinion that United States Patent No. 5,914,331 is eligible under 35 U.S.C. § 156 for an extension of its term. U.S. Patent No. 5,914,331 currently expires September 29, 2015 (terminally disclaimed). The length of extension calculated from § 156(g) is 886 days. The new term thus calculated is then subject to reduction to limit the extension to the fourteen-year period remaining in the term of the Patent under 35 U.S.C. § 156 (c)(3) from the date of NDA approval.

Applicants are thus of the opinion that United States Patent No. 5,914,331 is entitled to an extension of 642 days and a new patent term expiration date of July 2, 2017.

The length of extension of United States Patent No. 5,914,331 was determined according to the provisions of 35 U.S.C. § 156(c) and (g) as follows:

The length of extension is equal to the regulatory review period for the approved product which occurred after the patent issued, subject to the provisions of paragraphs (1) to (4) of 35 U.S.C. § 156(c), the regulatory review period defined in 35 U.S.C. § 156(g)(1), and the limitations of 35 U.S.C. § 156(g)(6).

The regulatory review period described in 35 U.S.C. § 156(g)(1)(B)(i) is the IND phase from September 20, 1997 to September 2, 2002. The period between the date that U.S. Patent No. 5,914,331 issued, June 22, 1999, and September 2, 2002, is 1168 days. The period of extension is one-half of this period (584 days) under 35 U.S.C. § 156(c)(2).

The regulatory review period described in 35 U.S.C. § 156(g)(1)(B)(ii) is the NDA phase from September 3, 2002 to July 2, 2003. This period of extension is 302 days.

The term of U.S. Patent No. 5,914,331 is eligible for extension under 35 U.S.C. § 156(c) for 584 days + 302 days = 886 days. This term is limited to 14 years remaining in the term of the patent under 35 U.S.C. § 156(c)(3) from the date of marketing approval.

Therefore, the extended term for which U.S. Patent No. 5,914,331 qualifies is 642 days, i.e. until July 2, 2017.

(13) Applicant acknowledges a duty to disclose to the Commissioner of Patents and Trademarks and the Secretary of Health and Human Services or the Secretary of

Agriculture any information which is material to the determination of entitlement to the extension sought.

(14) Authorization to charge the prescribed fee of $1120 for receiving and acting upon this application for extension to Deposit Account No. 07-1250 is enclosed.

(15) Correspondence relating to this application for patent term extension should be addressed to:

Intellectual Property Department
Gilead Sciences, Inc.
333 Lakeside Dr.
Foster City, CA 94404

The names and telephone numbers of the person(s) to whom inquiries may be made, at the above address, are:

Alex Andrus	Reg. No. 44,509	Tel. No. 650 522-5793
Mark Bosse	Reg. No. 35,071	Tel. No. 650 522-5569
William Schmonsees	Reg. No. 31,796	Tel. No. 650 522-5525

(b) Four additional copies of the application accompany this application.

Respectfully submitted,

William Schmonsees
Reg. No. 31,796
Date: 08/29/03

09-02-03

DAC

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:	)	
Liotta et al.	)	Attorney Docket No. EMU108D
Patent No.: 5,914,331	)	Group Art Unit: 1623
Issue Date: June 22, 1999	)	Customer No.: 25000

Title: ANTIVIRAL ACTIVITY AND RESOLUTION OF 2-HYDROXYMETHYL -5-(5-FLUOROCYTOSIN-1-YL)-1,3-OXATHIOLANE

TRANSMITTAL LETTER

Mail Stop Patent Extension
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

The following documents are transmitted in quintuplicate herewith for the above U.S. Patent:

- X Application for Extension of Patent Term
- X Exhibit A – Associate Power of Attorney
- X Exhibit B – Approved Package Insert
- X Exhibit C – Copy of U.S. Patent No. 5,914,331
- X Exhibit D – Copies of Terminal Disclaimers (4)
- X Exhibit E – Copy of Certificate of Correction
- X Exhibit F – Copy of Maintenance Fee Payment Receipt
- X Exhibit G – Description of Significant Activities by Marketing Applicant
- X Exhibit H – Letter Authorizing Reliance of Regulatory Submissions

The Commissioner is hereby authorized to charge the prescribed fee of $1120, and any additional fees which may be required, or to credit any overpayment to Deposit Account No. 07-1250.

Respectfully submitted,

By: _____
William Schmonsees, Reg. No. 31,796

GILEAD SCIENCES, INC.
333 Lakeside Drive
Foster City, CA 94404
Phone: (650) 522-5525
Fax: (650) 522-5575

I hereby certify that this correspondence is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated below and is addressed to the Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Express Mail Number: EL939648538US

August 29, 2003
Date

Robin Torres
Robin Torres

Attorney Docket No. EMU 108 Div(1)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Dennis C. Liotta, Raymond F. Schinazi and Woo-Baeg Choi

Patent No.: 5,914,331 (Serial No. 08/488,097) Art Unit: 1623

Issued: June 22, 1999 (Filed: June 7, 1995) Customer No. 25000

For: ANTIVIRAL ACTIVITY AND RESOLUTION OF 2-HYDROXYMETHYL-5-(5-FLUOROCYTOSIN-1-YL)-1,3-OXATHIOLANE

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

ASSOCIATE POWER OF ATTORNEY (37 CFR 1.34) AND CHANGE OF ATTORNEY'S ADDRESS IN APPLICATION

Please recognize as Associate Attorneys in this case for the limited purpose of matters related to the application for the extension of the term of this patent pursuant to 35 U.S.C. § 156.

Name of Attorney:	Alex Andrus	Reg. No. 44,509	Tel. No. 650-522-5793
Name of Attorney:	Mark L. Bosse	Reg. No. 35,071	Tel. No. 650-522-5569
Name of Attorney:	William Schmonsees	Reg. No. 31,796	Tel. No. 650-522-5525

Please direct all communications to:
GILEAD SCIENCES, INC.
Intellectual Property Dept.
333 Lakeside Drive
Foster City, CA 94404
Facsimile: 650-522-5575

By: Mary L. Severson

Mary L. Severson, Ph.D., J.D.
Assistant Vice President and Director
Office of Technology Transfer
EMORY UNIVERSITY
1784 North Decatur Road, Suite 130
Atlanta, GA 30322

CERTIFICATION OF FACSIMILE TRANSMISSION

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) are being facsimile transmitted to the Commissioner for Patents, P. O. Box 1450, Alexandria, VA 22313-1450.

VICKI COLLINS
(Type or print name of person mailing paper)

Date: August 25, 2003

Vicki Collins
(Signature of person mailing paper)

PTO/SB/96 (08-03)
Approved for use through 07/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office, U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number

STATEMENT UNDER 37 CFR 3.73(b)

Applicant/Patent Owner: Dennis C. Liotta, Raymond F. Schinazi and Woo-Baeg Choi

Application No./Patent No.: 5,914,331 Filed/Issue Date: June 22, 1999

Entitled: Antiviral Activity and Resolution of 2-Hydroxymethyl-5-(5-Fluorocytosin-1-yl)-1,3-Oxathiolane

Emory University, a University
(Name of Assignee) (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that it is:

1. [X] the assignee of the entire right, title, and interest; or

2. [ ] an assignee of less than the entire right, title and interest.
The extent (by percentage) of its ownership interest is _____ %
in the patent application/patent identified above by virtue of either:

A. [X] An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel 6128 Frame 0089, or for which a copy thereof is attached.
6128 0085
6128 0093

OR

B. [ ] A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as shown below:

1. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at Reel _____, Frame _____, or for which a copy thereof is attached.

2. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at Reel _____, Frame _____, or for which a copy thereof is attached.

3. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at Reel _____, Frame _____, or for which a copy thereof is attached.

[ ] Additional documents in the chain of title are listed on a supplemental sheet.

[ ] Copies of assignments or other documents in the chain of title are attached.
[NOTE: A separate copy (i.e., the original assignment document or a true copy of the original document) must be submitted to Assignment Division in accordance with 37 CFR Part 3, if the assignment is to be recorded in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

08/25/03
Date

(404) 727-7218
Telephone number

Mary L. Severson, Ph.D., J.D.
Typed or printed name

Signature

Assistant Vice President and Director, Office of Technology Transfer
Title

Emtriva™ (emtricitabine) Capsules

℞ Only

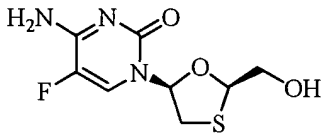
WARNING: LACTIC ACIDOSIS AND SEVERE HEPATOMEGALY WITH STEATOSIS, INCLUDING FATAL CASES, HAVE BEEN REPORTED WITH THE USE OF NUCLEOSIDE ANALOGUES ALONE OR IN COMBINATION WITH OTHER ANTIRETROVIRALS (SEE WARNINGS).

DESCRIPTION

EMTRIVA is the brand name of emtricitabine, a synthetic nucleoside analogue with activity against human immunodeficiency virus type 1 (HIV-1) reverse transcriptase.

The chemical name of emtricitabine is 5-fluoro-1-(2*R*,5*S*)-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine. Emtricitabine is the (-) enantiomer of a thio analogue of cytidine, which differs from other cytidine analogues in that it has a fluorine in the 5-position.

It has a molecular formula of $C_8H_{10}FN_3O_3S$ and a molecular weight of 247.24. It has the following structural formula:



Emtricitabine is a white to off-white powder with a solubility of approximately 112 mg/mL in water at 25 °C. The log P for emtricitabine is –0.43 and the pKa is 2.65.

EMTRIVA capsules are for oral administration. Each capsule contains 200 mg of emtricitabine and the inactive ingredients, crospovidone, magnesium stearate, microcrystalline cellulose and povidone.

MICROBIOLOGY

Mechanism of Action:

Emtricitabine, a synthetic nucleoside analog of cytosine, is phosphorylated by cellular enzymes to form emtricitabine 5'-triphosphate. Emtricitabine 5'-triphosphate inhibits the activity of the HIV-1 reverse transcriptase by competing with the natural substrate deoxycytidine 5'-triphosphate and by being incorporated into nascent viral DNA which results in chain termination. Emtricitabine 5'-triphosphate is a weak inhibitor of mammalian DNA polymerase α, β, ε and mitochondrial DNA polymerase γ.

Antiviral Activity In Vitro:

The *in vitro* antiviral activity of emtricitabine against laboratory and clinical isolates of HIV was assessed in lymphoblastoid cell lines, the MAGI-CCR5 cell line, and peripheral blood mononuclear cells. The 50% inhibitory concentration (IC_{50}) value for emtricitabine was in the range of 0.0013 to 0.64 μM (0.0003 to 0.158 μg/mL). In drug combination studies of emtricitabine with nucleoside reverse transcriptase inhibitors (abacavir, lamivudine, stavudine, tenofovir, zalcitabine, zidovudine), non-nucleoside reverse transcriptase inhibitors (delavirdine, efavirenz, nevirapine), and protease inhibitors (amprenavir, nelfinavir, ritonavir, saquinavir), additive to synergistic effects were observed. Most of these drug combinations have not been studied in humans. Emtricitabine displayed antiviral activity *in vitro* against HIV-1 clades A, C, D, E, F, and G (IC_{50} values ranged from 0.007 to 0.075 μM) and showed strain specific activity against HIV-2 (IC_{50} values ranged from 0.007 to 1.5 μM).

Drug Resistance:

Emtricitabine-resistant isolates of HIV have been selected *in vitro*. Genotypic analysis of these isolates showed that the reduced susceptibility to emtricitabine was associated with a mutation in the HIV reverse transcriptase gene at codon 184 which resulted in an amino acid substitution of methionine by valine or isoleucine (M184V/I).

Emtricitabine-resistant isolates of HIV have been recovered from some patients treated with emtricitabine alone or in combination with other antiretroviral agents. In a clinical study, viral isolates from 37.5% of treatment-naïve patients with virologic failure showed reduced susceptibility to emtricitabine. Genotypic analysis of these isolates showed that the resistance was due to M184V/I mutations in the HIV reverse transcriptase gene.

Cross Resistance:

Cross-resistance among certain nucleoside analogue reverse transcriptase inhibitors has been recognized. Emtricitabine-resistant isolates (M184V/I) were cross-resistant to lamivudine and zalcitabine but retained sensitivity to abacavir, didanosine, stavudine, tenofovir, zidovudine, and NNRTIs (delavirdine, efavirenz, and nevirapine). HIV-1 isolates containing the K65R mutation, selected *in vivo* by abacavir, didanosine, tenofovir, and zalcitabine, demonstrated reduced susceptibility to inhibition by emtricitabine. Viruses harboring mutations conferring reduced susceptibility to stavudine and zidovudine (M41L, D67N, K70R, L210W, T215Y/F, K219Q/E) or didanosine (L74V) remained sensitive to emtricitabine. HIV-1 containing the K103N mutation associated with resistance to NNRTIs was susceptible to emtricitabine.

CLINICAL PHARMACOLOGY

Pharmacodynamics:

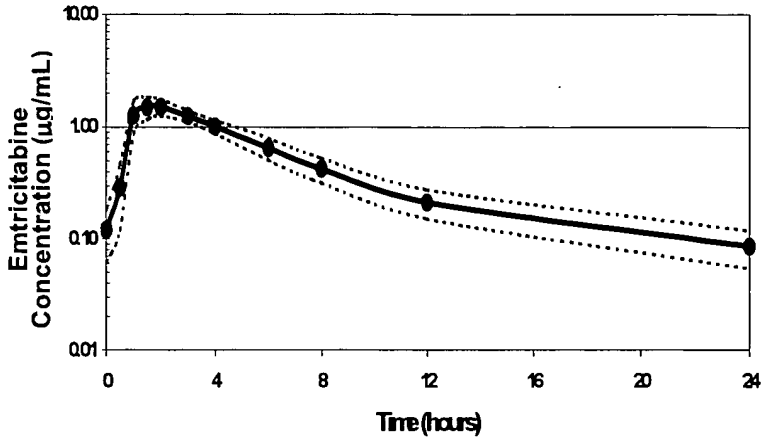
The *in vivo* activity of emtricitabine was evaluated in two clinical trials in which 101 patients were administered 25 to 400 mg a day of EMTRIVA as monotherapy for 10 to 14 days. A dose-related antiviral effect was observed, with a median decrease from baseline in plasma HIV-1 RNA of 1.3 $\log_{10}$ at a dose of 25 mg QD and 1.7 $\log_{10}$ to 1.9 $\log_{10}$ at a dose of 200 mg QD or BID.

Pharmacokinetics:

The pharmacokinetics of emtricitabine were evaluated in healthy volunteers and HIV-infected individuals. Emtricitabine pharmacokinetics are similar between these populations.

Figure 1 shows the mean steady-state plasma emtricitabine concentration-time profile in 20 HIV-infected subjects receiving EMTRIVA.

Figure 1. Mean (± 95% CI) Steady-State Plasma Emtricitabine Concentrations in HIV-Infected Adults (n = 20)



Absorption: Emtricitabine is rapidly and extensively absorbed following oral administration with peak plasma concentrations occurring at 1 to 2 hours post-dose. Following multiple dose oral administration of EMTRIVA to 20 HIV-infected subjects, the (mean ± SD) steady-state plasma emtricitabine peak concentration (C_{max}) was 1.8 ± 0.7 µg/mL and the area-under the plasma concentration-time curve over a 24-hour dosing interval (AUC) was 10.0 ± 3.1 hr*µg/mL. The mean steady state plasma trough concentration at 24 hours post-dose was 0.09 µg/mL. The mean absolute bioavailability of EMTRIVA was 93%.

The multiple dose pharmacokinetics of emtricitabine are dose proportional over a dose range of 25 to 200 mg.

Effects of Food on Oral Absorption: EMTRIVA may be taken with or without food. Emtricitabine systemic exposure (AUC) was unaffected while C_{max} decreased by 29% when EMTRIVA was administered with food (an approximately 1000 kcal high-fat meal).

Distribution: *In vitro* binding of emtricitabine to human plasma proteins was <4% and independent of concentration over the range of 0.02 – 200 µg/mL. At peak plasma concentration, the mean plasma to blood drug concentration ratio was ~ 1.0 and the mean semen to plasma drug concentration ratio was ~ 4.0.

Metabolism: *In vitro* studies indicate that emtricitabine is not an inhibitor of human CYP450 enzymes. Following administration of ^{14}C-emtricitabine, complete recovery of the dose was achieved in urine (~ 86%) and feces (~ 14%). Thirteen percent (13%) of the dose was recovered in urine as three putative metabolites. The biotransformation of emtricitabine includes oxidation of the thiol moiety to form the 3'-sulfoxide diastereomers (~ 9% of dose) and conjugation with glucuronic acid to form 2'-O-glucuronide (~ 4% of dose). No other metabolites were identifiable.

Elimination: The plasma emtricitabine half-life is approximately 10 hours.
The renal clearance of emtricitabine is greater than the estimated creatinine clearance, suggesting elimination by both glomerular filtration and active tubular secretion. There may be competition for elimination with other compounds that are also renally eliminated.

Special Populations:
The pharmacokinetics of emtricitabine were similar in male and female patients and no pharmacokinetic differences due to race have been identified.

The pharmacokinetics of emtricitabine have not been fully evaluated in children or in the elderly.

The pharmacokinetics of emtricitabine have not been studied in patients with hepatic impairment, however, emtricitabine is not metabolized by liver enzymes, so the impact of liver impairment should be limited.

The pharmacokinetics of emtricitabine are altered in patients with renal impairment (See PRECAUTIONS). In patients with creatinine clearance < 50 mL/min or with end-stage renal disease (ESRD) requiring dialysis, C_{max} and AUC of emtricitabine were increased due to a reduction in renal clearance (Table 1). It is recommended that the dosing interval for EMTRIVA be modified in patients with creatinine clearance < 50 mL/min or in patients with ESRD who require dialysis (see DOSAGE AND ADMINISTRATION).

Table 1. Mean ± SD Pharmacokinetic Parameters in Patients with Varying Degrees of Renal Function

Creatinine clearance (mL/min)	>80 (n=6)	50-80 (n=6)	30-49 (n=6)	<30 (n=5)	ESRD* <30 (n=5)
Baseline Creatinine clearance (mL/min)	107 ± 21	59.8 ± 6.5	40.9 ± 5.1	22.9 ± 5.3	8.8 ± 1.4
C_{max} (μg/mL)	2.2 ± 0.6	3.8 ± 0.9	3.2 ± 0.6	2.8 ± 0.7	2.8 ± 0.5
AUC (hr•μg/mL)	11.8 ± 2.9	19.9 ± 1.1	25.0 ± 5.7	34.0 ± 2.1	53.2 ± 9.9
CL/F (mL/min)	302 ± 94	168 ± 10	138 ± 28	99 ± 6	64 ± 12
CLr (mL/min)	213.3 ± 89.0	121.4 ± 39.0	68.6 ± 32.1	29.5 ± 11.4	-

*ESRD patients requiring dialysis
"-" = not applicable

Hemodialysis: Hemodialysis treatment removes approximately 30% of the emtricitabine dose over a 3-hour dialysis period starting within 1.5 hours of emtricitabine dosing (blood flow rate of 400 mL/min and a dialysate flow rate of 600 mL/min). It is not known whether emtricitabine can be removed by peritoneal dialysis.

Drug Interactions

At concentrations up to 14 fold higher than those observed *in vivo*, emtricitabine did not inhibit *in vitro* drug metabolism mediated by any of the following human CYP 450 isoforms: CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6 and CYP3A4. Emtricitabine did not inhibit the enzyme responsible for glucuronidation (uridine-5'-disphosphoglucuronyl transferase). Based on the results of these *in vitro* experiments and the known elimination pathways of emtricitabine, the potential for CYP450 mediated interactions involving emtricitabine with other medicinal products is low.

EMTRIVA has been evaluated in healthy volunteers in combination with tenofovir disoproxil fumarate (DF), indinavir, famciclovir, and stavudine. Tables 2 and 3 summarize the pharmacokinetic effects of co-administered drug on emtricitabine pharmacokinetics and effects of emtricitabine on the pharmacokinetics of co-administered drug.

Table 2. Drug Interactions: Change in Pharmacokinetic Parameters for Emtricitabine in the Presence of the Co-administered Drug[1]

Co-Administered Drug	Dose of Co-Administered Drug (mg)	Emtricitabine Dose (mg)	N	% Change of Co-administered Drug Pharmacokinetic Parameters [2] (90% CI)		
				C_{max}	AUC	C_{min}
Tenofovir DF	300 once daily x 7 days	200 once daily x 7 days	17	⇔	⇔	↑ 20 (↑ 12 to ↑ 29)
Indinavir	800 x 1	200 x 1	12	⇔	⇔	-
Famciclovir	500 x 1	200 x 1	12	⇔	⇔	-
Stavudine	40 x 1	200 x 1	6	⇔	⇔	-

1. All interaction studies conducted in healthy volunteers
2. ↑ = Increase; ↓ = Decrease; ⇔ = no effect; "-" = not applicable

Table 3. Drug Interactions: Change in Pharmacokinetic Parameters for Co-administered Drug in the Presence of Emtricitabine[1]

Co-Administered Drug	Dose of Co-Administered Drug (mg)	Emtricitabine Dose (mg)	N	% Change of Co-administered Drug Pharmacokinetic Parameters[2] (90% CI)		
				C_{max}	AUC	C_{min}
Tenofovir DF	300 once daily x 7 days	200 once daily x 7 days	17	⇔	⇔	⇔
Indinavir	800 x 1	200 x 1	12	⇔	⇔	-
Famciclovir	500 x 1	200 x 1	12	⇔	⇔	-
Stavudine	40 x 1	200 x 1	6	⇔	⇔	-

1. All interaction studies conducted in healthy volunteers
2. ↑ = Increase; ↓ = Decrease; ⇔ = no effect; "-" = not applicable

INDICATION AND USAGE

EMTRIVA is indicated, in combination with other antiretroviral agents, for the treatment of HIV-1 infection in adults.

This indication is based on analyses of plasma HIV-1 RNA levels and CD4 cell counts from controlled studies of 48 weeks duration in antiretroviral-naïve patients and antiretroviral-treatment-experienced patients who were virologically suppressed on an HIV treatment regimen.

In antiretroviral-treatment-experienced patients, the use of EMTRIVA may be considered for adults with HIV strains that are expected to be susceptible to EMTRIVA as assessed by genotypic or phenotypic testing. (See MICROBIOLOGY, Drug Resistance and Cross Resistance).

Description of Clinical Studies

Study 301A: EMTRIVA QD + didanosine QD + efavirenz QD compared to stavudine BID + didanosine QD + efavirenz QD

Study 301A was a 48 week double-blind, active-controlled multicenter study comparing EMTRIVA (200 mg QD) administered in combination with didanosine and efavirenz versus stavudine, didanosine and efavirenz in 571 antiretroviral naïve patients. Patients had a mean age of 36 years (range 18 to 69), 85% were male, 52% Caucasian, 16% African-American and 26% Hispanics. Patients had a mean baseline CD4 cell count of 318 cells/mm^3 (range 5-1317) and a median baseline plasma HIV RNA of 4.9 log_{10} copies/mL (range 2.6-7.0). Thirty-eight percent of patients had baseline viral loads > 100,000 copies/mL and 31% had CD4 cell counts < 200 cells/mL. Treatment outcomes are presented in Table 4 below.

Table 4. Outcomes of Randomized Treatment at Week 48 (Study 301A)

Outcome at Week 48	EMTRIVA+ didanosine+ efavirenz (N=286)	Stavudine+ didanosine+ efavirenz (N=285)
Responder[1]	81% (78%)	68% (59%)
Virologic Failure[2]	3%	11%
Death	0%	<1%
Study Discontinuation Due to Adverse Event	7%	13%
Study Discontinuation For Other Reasons[3]	9%	8%

1. Patients achieved and maintained confirmed HIV RNA < 400 copies/mL (<50 copies/mL) through Week 48.
2. Includes patients who failed to achieve virologic suppression or rebounded after achieving virologic suppression.
3. Includes lost to follow-up, patient withdrawal, non-compliance, protocol violation and other reasons.

The mean increase from baseline in CD4 cell count was 168 cells/mm^3 for the EMTRIVA arm and 134 cells/mm^3 for the stavudine arm.

Through 48 weeks in the EMTRIVA group, 5 patients (1.7%) experienced a new CDC Class C event, compared to 7 patients (2.5%) in the stavudine group.

Study 303: EMTRIVA QD + Stable Background Therapy (SBT) compared to lamivudine BID + SBT

Study 303 was a 48 week, open-label, active-controlled multicenter study comparing EMTRIVA (200 mg QD) to lamivudine, in combination with stavudine or zidovudine and a protease inhibitor or NNRTI in 440 patients who were on a lamivudine-containing triple-antiretroviral drug regimen for at least 12 weeks prior to study entry and had HIV-1 RNA ≤400 copies/mL.

Patients were randomized 1:2 to continue therapy with lamivudine (150 mg BID) or to switch to EMTRIVA (200 mg QD). All patients were maintained on their stable background regimen. Patients had a mean age of 42 years (range 22-80), 86% were male, 64% Caucasian, 21% African-American and 13% Hispanic. Patients had a mean baseline CD4 cell count of 527 cells/mm^3 (range 37-1909), and a median baseline plasma HIV RNA of 1.7 $\log_{10}$ copies/mL (range 1.7-4.0).

The median duration of prior antiretroviral therapy was 27.6 months.

Table 5. Outcomes of Randomized Treatment at Week 48 (Study 303)

Outcome at Week 48	EMTRIVA + ZDV/d4T + NNRTI/PI (N=294)	Lamivudine + ZDV/d4T + NNRTI/PI (N=146)
Responder[1]	77% (67%)	82% (72%)
Virologic Failure[2]	7%	8%
Death	0%	<1%
Study Discontinuation Due to Adverse Event	4%	0%
Study Discontinuation For Other Reasons[3]	12%	10%

1. Patients achieved and maintained confirmed HIV RNA < 400 copies/mL (< 50/mL) through Week 48.
2. Includes patients who failed to achieve virologic suppression or rebounded after achieving virologic suppression.
3. Includes lost to follow-up, patient withdrawal, non-compliance, protocol violation and other reasons.

The mean increase from baseline in CD4 cell count was 29 cells/mm^3 for the EMTRIVA arm and 61 cells/mm^3 for the lamivudine arm.

Through 48 weeks, in the EMTRIVA group 2 patients (0.7%) experienced a new CDC Class C event, compared to 2 patients (1.4%) in the lamivudine group.

CONTRAINDICATIONS

EMTRIVA is contraindicated in patients with previously demonstrated hypersensitivity to any of the components of the products.

WARNINGS

Lactic Acidosis/Severe Hepatomegaly with Steatosis

Lactic acidosis and severe hepatomegaly with steatosis, including fatal cases, have been reported with the use of nucleoside analogues alone or in combination, including emtricitabine and other antiretrovirals. A majority of these cases have been in women. Obesity and prolonged nucleoside exposure may be risk factors. However, cases have also been reported in patients with no known risk factors. Treatment with EMTRIVA should be suspended in any patient who develops clinical or laboratory findings suggestive of lactic acidosis or pronounced hepatotoxicity (which may include hepatomegaly and steatosis even in the absence of marked transaminase elevations).

Post Treatment Exacerbation of Hepatitis

It is recommended that all patients with HIV be tested for the presence of chronic hepatitis B virus (HBV) before initiating antiretroviral therapy. EMTRIVA is not indicated for the treatment of chronic HBV infection and the safety and efficacy of EMTRIVA have not been established in patients co-infected with HBV and HIV. Exacerbations of hepatitis B have been reported in patients after the discontinuation of EMTRIVA. Patients co-infected with HIV and HBV should be closely monitored with both clinical and laboratory follow-up for at least several months after stopping treatment.

PRECAUTIONS

Patients with Impaired Renal Function

Emtricitabine is principally eliminated by the kidney. Reduction of the dosage of EMTRIVA is recommended for patients with impaired renal function (see CLINICAL PHARMACOLOGY and DOSAGE AND ADMINISTRATION).

Drug Interactions

The potential for drug interactions with EMTRIVA has been studied in combination with indinavir, stavudine, famciclovir, and tenofovir disoproxil fumarate. There were no clinically significant drug interactions for any of these drugs (see CLINICAL PHARMACOLOGY, Drug Interactions).

Fat Redistribution

Redistribution/accumulation of body fat including central obesity, dorsocervical fat enlargement (buffalo hump), peripheral wasting, facial wasting, breast enlargement, and "cushingoid appearance" have been observed in patients receiving antiretroviral therapy. The mechanism and long-term consequences of these events are unknown. A causal relationship has not been established.

Information for Patients

EMTRIVA is not a cure for HIV infection and patients may continue to experience illnesses associated with HIV infection, including opportunistic infections. Patients should remain under the care of a physician when using EMTRIVA.

Patients should be advised that:

- the use of EMTRIVA has not been shown to reduce the risk of transmission of HIV to others through sexual contact or blood contamination.
- the long term effects of EMTRIVA are unknown.
- EMTRIVA Capsules are for oral ingestion only.
- it is important to take EMTRIVA with combination therapy on a regular dosing schedule to avoid missing doses.
- redistribution or accumulation of body fat may occur in patients receiving antiretroviral therapy and that the cause and long-term health effects of these conditions are not known.

Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis: Long-term carcinogenicity studies of emtricitabine in rats and mice are in progress.

Mutagenesis: Emtricitabine was not genotoxic in the reverse mutation bacterial test (Ames test), mouse lymphoma or mouse micronucleus assays.

Impairment of Fertility: Emtricitabine did not affect fertility in male rats at approximately 140-fold or in male and female mice at approximately 60-fold higher exposures (AUC) than in humans given the recommended 200 mg daily dose. Fertility was normal in the offspring of mice exposed daily from before birth (in utero) through sexual maturity at daily exposures (AUC) of approximately 60-fold higher than human exposures at the recommended 200 mg daily dose.

Pregnancy
Pregnancy Category B
The incidence of fetal variations and malformations was not increased in embryofetal toxicity studies performed with emtricitabine in mice at exposures (AUC) approximately 60-fold higher and in rabbits at approximately 120-fold higher than human exposures at the recommended daily dose. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, EMTRIVA should be used during pregnancy only if clearly needed.

Antiretroviral Pregnancy Registry: To monitor fetal outcomes of pregnant women exposed to emtricitabine, an antiretroviral Pregnancy Registry has been established. Healthcare providers are encouraged to register patients by calling 1-800-258-4263.

Nursing Mothers: The Centers for Disease Control and Prevention recommend that HIV-infected mothers not breast-feed their infants to avoid risking postnatal transmission of HIV. It is not known whether emtricitabine is secreted into human milk. Because of both the potential for HIV transmission and the potential for serious adverse reactions in nursing infants, **mothers should be instructed not to breast-feed if they are receiving EMTRIVA.**

Pediatric Use:
Safety and effectiveness in pediatric patients have not been established.

Geriatric Use:
Clinical studies of EMTRIVA did not contain sufficient numbers of subjects aged 65 years and over to determine whether they respond differently from younger subjects. In general, dose selection for the elderly patient should be cautious, keeping in mind the greater frequency of decreased hepatic, renal, or cardiac function, and of concomitant disease or other drug therapy (see PRECAUTIONS: Patients with Impaired Renal Function and DOSAGE AND ADMINISTRATION).

ADVERSE REACTIONS

More than 2000 adult patients with HIV infection have been treated with EMTRIVA alone or in combination with other antiretroviral agents for periods of 10 days to 200 weeks in Phase I-III clinical trials.

Assessment of adverse reactions is based on data from studies 301A and 303 in which 571 treatment naïve (301A) and 440 treatment experienced (303) patients received EMTRIVA 200 mg (n=580) or comparator drug (n=431) for 48 weeks.

The most common adverse events that occurred in patients receiving EMTRIVA with other antiretroviral agents in clinical trials were headache, diarrhea, nausea, and rash, which were generally of mild to moderate severity. Approximately 1% of patients discontinued participation in the clinical studies due to these events. All adverse events were reported with similar frequency in EMTRIVA and control treatment groups with the exception of skin discoloration which was reported with higher frequency in the EMTRIVA treated group.
Skin discoloration, manifested by hyperpigmentation on the palms and/or soles was generally mild and asymptomatic. The mechanism and clinical significance are unknown.

A summary of EMTRIVA treatment emergent clinical adverse events in studies 301A and 303 is provided in Table 6 below.

Table 6. Selected Treatment-Emergent Adverse Events (All Grades, Regardless of Causality) Reported in ≥ 3% of EMTRIVA-Treated Patients in Either Study 301A or 303 (0-48 weeks)

Adverse event	303		301A	
	EMTRIVA + ZDV/d4T + NNRTI/PI (n=294)	Lamivudine + ZDV/d4T + NNRTI/PI (n=146)	EMTRIVA + didanosine + efavirenz (n=286)	Stavudine + didanosine + efavirenz (n=285)
Body as a Whole				
Abdominal Pain	8%	11%	14%	17%
Asthenia	16%	10%	12%	17%
Headache	13%	6%	22%	25%
Digestive System				
Diarrhea	23%	18%	23%	32%
Dyspepsia	4%	5%	8%	12%
Nausea	18%	12%	13%	23%
Vomiting	9%	7%	9%	12%
Musculoskeletal				
Arthralgia	3%	4%	5%	6%
Myalgia	4%	4%	6%	3%
Nervous System				
Abnormal dreams	2%	<1%	11%	19%
Depressive disorders	6%	10%	9%	13%
Dizziness	4%	5%	25%	26%
Insomnia	7%	3%	16%	21%
Neuropathy/Peripheral Neuritis	4%	3%	4%	13%
Paresthesia	5%	7%	6%	12%
Respiratory				
Increased cough	14%	11%	14%	8%
Rhinitis	18%	12%	12%	10%
Skin				
Rash event[1]	17%	14%	30%	33%

1. Rash event includes rash, pruritus, maculopapular rash, urticaria, vesiculobullous rash, pustular rash, and allergic reaction.

Laboratory Abnormalities:
Laboratory abnormalities in these studies occurred with similar frequency in the EMTRIVA and comparator groups. A summary of Grade 3 and 4 laboratory abnormalities is provided in Table 7 below.

Table 7. Treatment-Emergent Grade 3 / 4 Laboratory Abnormalities Reported in ≥ 1% of EMTRIVA-Treated Patients in Either Study 301A or 303

	303		301A	
Number of Patients Treated	**EMTRIVA + ZDV/d4T + NNRTI/PI (n=294)**	**Lamivudine + ZDV/d4T + NNRTI/PI (n=146)**	**EMTRIVA + didanosine + efavirenz (n=286)**	**Stavudine + didanosine + efavirenz (n=285)**
Percentage with Grade 3 or Grade 4 laboratory abnormality	31%	28%	34%	38%
ALT (>5.0 x ULN[1])	2%	1%	5%	6%
AST (>5.0 x ULN)	3%	<1%	6%	9%
Bilirubin (>2.5 x ULN)	1%	2%	<1%	<1%
Creatine kinase (>4.0 x ULN)	11%	14%	12%	11%
Neutrophils (<750 mm^3)	5%	3%	5%	7%
Pancreatic amylase (>2.0 x ULN)	2%	2%	<1%	1%
Serum amylase (>2.0 x ULN)	2%	2%	5%	10%
Serum glucose (<40 or >250 mg/dL)	3%	3%	2%	3%
Serum lipase (>2.0 x ULN)	<1%	<1%	1%	2%
Triglycerides (>750 mg/dL)	10%	8%	9%	6%

1. ULN=Upper limit of normal

OVERDOSAGE

There is no known antidote for EMTRIVA. Limited clinical experience is available at doses higher than the therapeutic dose of EMTRIVA. In one clinical pharmacology study single doses of emtricitabine 1200 mg were administered to 11 patients. No severe adverse reactions were reported.

The effects of higher doses are not known. If overdose occurs the patient should be monitored for signs of toxicity, and standard supportive treatment applied as necessary.

Hemodialysis treatment removes approximately 30% of the emtricitabine dose over a 3-hour dialysis period starting within 1.5 hours of emtricitabine dosing (blood flow rate of 400 mL/min and a dialysate flow rate of 600 mL/min). It is not known whether emtricitabine can be removed by peritoneal dialysis.

DOSAGE AND ADMINISTRATION

For adults 18 years of age and older, the dose of EMTRIVA is 200 mg once daily taken orally with or without food.

Dose Adjustment in Patients with Renal Impairment:
Significantly increased drug exposures were seen when EMTRIVA was administered to patients with renal impairment, **(see CLINICAL PHARMACOLOGY: Special Populations)**. Therefore, the dosing interval of EMTRIVA should be adjusted in patients with baseline creatinine clearance < 50 mL/min using the following guidelines (see Table 8). The safety and effectiveness of these dosing interval adjustment guidelines have not been clinically evaluated. Therefore, clinical response to treatment and renal function should be closely monitored in these patients.

Table 8. Dosing Interval Adjustment in Patients with Renal Impairment

	Creatinine Clearance (mL/min)			
	≥ 50	30 - 49	15 - 29	< 15 (including patients requiring hemodialysis)*
Recommended Dose and Dosing Interval	200 mg every 24 hours	200 mg every 48 hours	200 mg every 72 hours	200 mg every 96 hours

* Hemodialysis Patients: If dosing on day of dialysis, give dose after dialysis.

HOW SUPPLIED

EMTRIVA is available as capsules. EMTRIVA capsules, 200 mg, are size 1 hard gelatin capsules with a blue cap and white body, printed with "200 mg" in black on the cap and "GILEAD" and the corporate logo in black on the body.

They are packaged in bottles of 30 capsules (NDC 61958-0601-1) with induction sealed child-resistant closures.

Store at 25 °C (77 °F); excursions permitted to 15 °C – 30 °C (59 °F – 86 °F) [see USP Controlled Room Temperature].

EMTRIVA is manufactured for Gilead Sciences, Inc., Foster City, CA 94404.

July 2003

RM-1466

Patient Information
EMTRIVA™ (em-treev'-ah) Capsules
Generic name: emtricitabine (em tri SIT uh bean)

Read the Patient Information that comes with EMTRIVA before you start using it and each time you get a refill. There may be new information. This information does not take the place of talking to your healthcare provider about your medical condition or treatment. You should stay under a healthcare provider's care when taking EMTRIVA. **Do not change or stop your medicine without first talking with your healthcare provider.** Talk to your healthcare provider or pharmacist if you have any questions about EMTRIVA.

What is the most important information I should know about EMTRIVA?

- **Some people who have taken medicines like EMTRIVA (a nucleoside analog) have developed a serious condition called lactic acidosis** (buildup of an acid in the blood). Lactic acidosis can be a medical emergency and may need to be treated in the hospital. **Call your healthcare provider right away if you get the following signs of lactic acidosis.**

- You feel very weak or tired.
- You have unusual (not normal) muscle pain.
- You have trouble breathing.
- You have stomach pain with nausea and vomiting.
- You feel cold, especially in your arm and legs.
- You feel dizzy or lightheaded.
- You have a fast or irregular heartbeat.

- **Some people who have taken medicines like EMTRIVA have developed serious liver problems called hepatotoxicity**, with liver enlargement (hepatomegaly) and fat in the liver (steatosis). **Call your healthcare provider right away if you get the following signs of liver problems.**

- Your skin or the white part of your eyes turns yellow (jaundice).
- Your urine turns dark.
- Your bowel movements (stools) turn light in color.
- You don't feel like eating food for several days or longer.
- You feel sick to your stomach (nausea).
- You have lower stomach area (abdominal) pain.

- **You may be more likely to get lactic acidosis or liver problems** if you are female, very overweight (obese), or have been taking nucleoside analog medicines, like EMTRIVA, for a long time.

- **EMTRIVA is not for the treatment of Hepatitis B Virus (HBV) infection.** Patients with both HBV and human immunodeficiency virus (HIV) infection who take EMTRIVA need close medical follow-up for several months after stopping treatment with EMTRIVA. Follow-up includes medical exams and blood tests to check for HBV that is getting worse. **Patients with HBV infection, who take EMTRIVA and then stop it, may get "flare-ups" of their hepatitis. A "flare-up" is when the disease suddenly returns in a worse way than before.**

What is EMTRIVA?

EMTRIVA is a type of medicine called an HIV (human immunodeficiency virus) nucleoside reverse transcriptase inhibitor (NRTI). EMTRIVA is always used with other anti-HIV medicines to treat people with HIV infection. EMTRIVA is for adults age 18 and older. EMTRIVA has not been studied fully in children under age 18 or adults over age 65.

HIV infection destroys CD4 (T) cells, which are important to the immune system. The immune system helps fight infection. After a large number of T cells are destroyed, acquired immune deficiency syndrome (AIDS) develops.

EMTRIVA helps to block HIV reverse transcriptase, a chemical in your body (enzyme) that is needed for HIV to multiply. EMTRIVA may lower the amount of HIV in the blood (viral load). EMTRIVA may also help to increase the number of T cells called CD4 cells. Lowering the amount of HIV in the blood lowers the chance of death or infections that happen when your immune system is weak (opportunistic infections).

EMTRIVA does not cure HIV infection or AIDS. The long-term effects of EMTRIVA are not known at this time. People taking EMTRIVA may still get opportunistic infections or other conditions that happen with HIV infection. Opportunistic infections are infections that develop because the immune system is weak. Some of these conditions are pneumonia, herpes virus infections, and *Mycobacterium avium* complex (MAC) infections. **It is very important that you see your healthcare provider regularly while taking EMTRIVA.**

EMTRIVA does not lower your chance of passing HIV to other people through sexual contact, sharing needles, or being exposed to your blood. For your health and the health of others, it is important to always practice safer sex by using a latex or polyurethane condom or other barrier to lower the chance of sexual contact with semen, vaginal secretions, or blood. Never use or share dirty needles.

Who should not take EMTRIVA?

Do not take EMTRIVA if you are allergic to EMTRIVA or any of its ingredients. The active ingredient is emtricitabine. See the end of this leaflet for a complete list of ingredients.

What should I tell my healthcare provider before taking EMTRIVA?

Tell your healthcare provider

- **If you are pregnant or planning to become pregnant.** We do not know if EMTRIVA can harm your unborn child. You and your healthcare provider will need to decide if EMTRIVA is right for you. If you use EMTRIVA while you are pregnant, talk to your healthcare provider about how you can be on the EMTRIVA Antiviral Pregnancy Registry.

- **If you are breast-feeding.** You should not breast feed if you are HIV-positive because of the chance of passing the HIV virus to your baby. Also, it is not known if EMTRIVA can pass into your breast milk and if it can harm your baby. If you are a woman who has or will have a baby, talk with your healthcare provider about the best way to feed your baby.

- **If you have kidney problems.** You may need to take EMTRIVA less often.

- **If you have any liver problems including Hepatitis B Virus infection.**

- **Tell your healthcare provider about all your medical conditions.**

- **Tell your healthcare provider about all the medicines you take** such as prescription and non-prescription medicines and dietary supplements. Keep a complete list of all the medicines that you take. Make a new list when medicines are added or stopped. Give copies of this list to all of your healthcare providers and pharmacist **every** time you visit or fill a prescription.

How should I take EMTRIVA?

- Take EMTRIVA every day by mouth exactly as your healthcare provider prescribed it. Follow the directions from your healthcare provider, exactly as written on the label.
- The usual dose of EMTRIVA is 1 capsule once a day. EMTRIVA is always used with other anti-HIV medicines.
- EMTRIVA may be taken with or without a meal. Food does not affect how EMTRIVA works.
- If you forget to take EMTRIVA, take it as soon as you remember that day. **Do not** take more than 1 dose of EMTRIVA in a day. **Do not** take 2 doses at the same time. Call your healthcare provider or pharmacist if you are not sure what to do. **It is important that you do not miss any doses of EMTRIVA or your other anti-HIV medicines.**
- When your EMTRIVA supply starts to run low, get more from your healthcare provider or pharmacy. This is very important because the amount of virus in your blood may increase if the medicine is stopped for even a short time. The virus may develop resistance to EMTRIVA and become harder to treat.
- Stay under a healthcare provider's care when taking EMTRIVA. Do not change your treatment or stop treatment without first talking with your healthcare provider.
- If you take too much EMTRIVA, call your local poison control center or emergency room right away.

What should I avoid while taking EMTRIVA?

- **Do not breast-feed.** See "What should I tell my healthcare provider before taking EMTRIVA?" Talk with your healthcare provider about the best way to feed your baby.
- **Avoid doing things that can spread HIV infection** since EMTRIVA doesn't stop you from passing the HIV infection to others.
- **Do not share needles or other injection equipment.**
- **Do not share personal items that can have blood or body fluids on them, like toothbrushes or razor blades.**
- **Do not have any kind of sex without protection.** Always practice safer sex by using a latex or polyurethane condom or other barrier to reduce the chance of sexual contact with semen, vaginal secretions, or blood.

What are the possible side effects of EMTRIVA?

EMTRIVA may cause the following serious side effects (see "What is the most important information I should know about EMTRIVA?"):

- **lactic acidosis** (buildup of an acid in the blood). Lactic acidosis can be a medical emergency and may need to be treated in the hospital. **Call your doctor right away if you get signs of lactic acidosis.** (See "What is the most important information I should know about EMTRIVA?")

- **serious liver problems (hepatotoxicity)**, with liver enlargement (hepatomegaly) and fat in the liver (steatosis). Call your healthcare provider right away if you get any signs of liver problems. (See "What is the most important information I should know about EMTRIVA?")

- **"flare-ups" of hepatitis B virus infection,** in which the disease suddenly returns in a worse way than before, can occur if you stop taking EMTRIVA. EMTRIVA is not for the treatment of Hepatitis B Virus (HBV) infection.

Other side effects with EMTRIVA when used with other anti-HIV medicines Include:

- Changes in body fat have been seen in some patients taking EMTRIVA and other anti-HIV medicines. These changes may include increased amount of fat in the upper back and neck ("buffalo hump"), breast, and around the main part of your body (trunk). Loss of fat from the legs, arms and face may also happen. The cause and long term health effects of these conditions are not known at this time.

The most common side effects of EMTRIVA used with other anti-HIV medicines are headache, diarrhea, nausea and rash. Skin discoloration may also happen with EMTRIVA.

There have been other side effects in patients taking EMTRIVA. However, these side effects may have been due to other medicines that patients were taking or to HIV itself. Some of these side effects can be serious.

This list of side effects is **not** complete. If you have questions about side effects, ask your healthcare provider or pharmacist. You should report any new or continuing symptoms to your healthcare provider right away. Your healthcare provider may be able to help you manage these side effects.

How do I store EMTRIVA?

- **Keep EMTRIVA and all other medicines out of reach of children.**
- Store EMTRIVA at between 59 °F and 86 °F (15 °C to 30 °C). Do not keep your medicine in places that are too hot or cold.
- Do not keep medicine that is out of date or that you no longer need. If you throw any medicines away make sure that children will not find them.

General information about EMTRIVA:

Medicines are sometimes prescribed for conditions that are not mentioned in patient information leaflets. Do not use EMTRIVA for a condition for which it was not prescribed. Do not give EMTRIVA to other people, even if they have the same symptoms you have. It may harm them.

This leaflet summarizes the most important information about EMTRIVA. If you would like more information, talk with your doctor. You can ask your healthcare provider or pharmacist for information about EMTRIVA that is written for health professionals. For more information, you may also call 1-800-GILEAD5.

What are the ingredients of EMTRIVA?

Active Ingredient: emtricitabine

Inactive Ingredients: crospovidone, magnesium stearate, microcrystalline cellulose, and povidone.

Rx Only

July 2003

RM-1466

US005914331A

United States Patent [19]

Liotta et al.

[11] Patent Number: 5,914,331

[45] Date of Patent: *Jun. 22, 1999

[54] **ANTIVIRAL ACTIVITY AND RESOLUTION OF 2-HYDROXYMETHYL-5-(5-FLUOROCYTOSIN-1-YL)-1,3-OXATHIOLANE**

[75] Inventors: **Dennis C. Liotta**, Stone Mountain; **Raymond F. Schinazi**, Decatur, both of Ga.; **Woo-Baeg Choi**, North Brunswick, N.J.

[73] Assignee: **Emory University**, Atlanta, Ga.

[*] Notice: This patent is subject to a terminal disclaimer.

[21] Appl. No.: **08/488,097**

[22] Filed: **Jun. 7, 1995**

Related U.S. Application Data

[63] Continuation of application No. 07/831,153, Feb. 12, 1992, which is a continuation-in-part of application No. 07/659,760, Feb. 22, 1991, Pat. No. 5,210,085, which is a continuation-in-part of application No. 07/473,318, Feb. 1, 1990, Pat. No. 5,204,466, and a continuation-in-part of application No. 07/736,089, Jul. 26, 1991, abandoned, and a continuation-in-part of application No. 07/659,760, Feb. 22, 1991, Pat. No. 5,210,085.

[51] **Int. Cl.**[6] **A01N 43/54**; A61K 31/70; C07D 239/02

[52] **U.S. Cl.** **514/274**; 514/49; 514/50; 514/51; 514/885; 544/317

[58] **Field of Search** 544/317; 514/274, 514/49, 50, 51, 888

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,000,137	12/1976	Dvonch et al.	260/252
4,336,381	6/1982	Nagata et al.	544/313
4,861,759	8/1989	Hiroaki et al.	514/46
4,879,277	11/1989	Mitsuya et al.	514/49
4,900,828	2/1990	Belica et al.	544/317
4,916,122	4/1990	Chu et al.	514/50
4,963,533	10/1990	de Clercq et al.	514/49
5,011,774	4/1991	Farina et al.	435/87
5,041,449	8/1991	Belleau et al.	514/274
5,041,499	8/1991	Belleau et al.	514/274
5,047,407	9/1991	Belleau et al.	514/274
5,059,690	10/1991	Zahler et al.	544/276
5,071,983	12/1991	Koszalka et al.	544/317
5,179,104	1/1993	Chu et al.	544/310
5,185,437	2/1993	Koszalka et al.	536/24
5,204,466	4/1993	Liotta et al.	544/317
5,210,085	5/1993	Liotta et al.	514/274
5,234,913	8/1993	Furman, Jr. et al.	514/49
5,248,776	9/1993	Chu et al.	544/310
5,270,315	12/1993	Belleau et al.	514/262
5,276,151	1/1994	Liotta	544/317
5,444,063	8/1995	Schinazi	514/262
5,466,805	11/1995	Belleau et al. .	
5,466,806	11/1995	Belleau et al.	544/310
5,486,520	1/1996	Belleau et al. .	
5,532,246	7/1996	Belleau et al. .	
5,538,975	7/1996	Dionne .	
5,539,116	7/1996	Liotta et al.	544/317
5,587,480	12/1996	Belleau et al.	544/310
5,618,820	4/1997	Dionne .	
7,686,617	7/1996	Cheng et al. .	
7,718,806	6/1991	Cheng .	
7,785,545	10/1991	Cheng .	

FOREIGN PATENT DOCUMENTS

665187	2/1992	Australia .
630913	9/1992	Australia .
0 217 580	4/1987	European Pat. Off. .
0 337 713	10/1988	European Pat. Off. .
0 375 329	1/1990	European Pat. Off. .
350 811	1/1990	European Pat. Off. .
357 009	3/1990	European Pat. Off. .
0 361 831	4/1990	European Pat. Off. .
0 382 526	6/1990	European Pat. Off. .
0 433 898	8/1990	European Pat. Off. .
382526	8/1990	European Pat. Off. .
421 636	4/1991	European Pat. Off. .
0 494 119	7/1992	European Pat. Off. .
0 515 144	11/1992	European Pat. Off. .
0 515 156	11/1992	European Pat. Off. .
0 515 157	11/1992	European Pat. Off. .
0 526 253	2/1993	European Pat. Off. .
2-29469	3/1990	Japan .
2-69476	3/1990	Japan .
07109221	4/1995	Japan .

(List continued on next page.)

OTHER PUBLICATIONS

Abobo, et al., "Pharmacokinetics of 2',3'–Dideoxy–5–fluoro–3'–thiacytidine in Rats," *J. of Pharmaceutical Sciences,* 83(1):96–99 (1994).

Agranat and Biedermann, "Intellectual Property and Chirality: Patentability of Enantiomers of Racemic Drugs in a Racemic Switch Scenario," *8th Chirality Conference, Edinburgh, UK* (Jul. 2, 1996).

Balzarini, J., et al., "Potent and Selective Anti–HTLV–III/LAV Activity of 2',3'–Dideoxycytidinene, the 2',3'–Unsaturated Derivative of 2', 3'–Dideoxycytidine," *Biochemical and Biophysical Research Communications,* 140(2): 735–742 (1986).

Baschang, et al., "The enantiomers of 1.beta.–adenyl–2.alpha.–hydroxy–3.beta.–(hydroxymethyl)cyclobutane," *Tetrahedron:Asymmetry,* 3(2):193–6 (1992).

(List continued on next page.)

Primary Examiner—James O. Wilson

Attorney, Agent, or Firm—Sherry M. Knowles; Jacqueline Haley; King & Spalding

[57] **ABSTRACT**

A method and composition for the treatment of HIV and HBV infections in humans is disclosed that includes administering an effective amount of 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane, a pharmaceutically acceptable derivative thereof, including a 5' or N^4 alkylated or acylated derivative, or a pharmaceutically acceptable salt thereof, in a pharmaceutically acceptable carrier.

A process for the resolution of a racemic mixture of nucleoside enantiomers is also disclosed that includes the step of exposing the racemic mixture to an enzyme that preferentially catalyzes a reaction in one of the enantiomers.

7 Claims, 6 Drawing Sheets

FOREIGN PATENT DOCUMENTS

8901258	12/1990	Netherlands .
238017	6/1994	New Zealand .
WO88/07532	10/1988	WIPO .
90/12023	10/1990	WIPO .
WO90/12023	10/1990	WIPO .
WO91/11186	8/1991	WIPO .
WO91/17159	11/1991	WIPO .
WO92/08727	5/1992	WIPO .
WO92/10496	6/1992	WIPO .
WO92/10497	6/1992	WIPO .
WO92/14729	9/1992	WIPO .
WO92/14743	9/1992	WIPO .
WO92/15309	9/1992	WIPO .
WO9215308	9/1992	WIPO .
WO92/18517	10/1992	WIPO .
WO92/21676	12/1992	WIPO .
WO94/04154	3/1994	WIPO .
WO94/09793	5/1994	WIPO .
WO94/14802	7/1994	WIPO .

OTHER PUBLICATIONS

Belleau, B., et al., "Design and Activity of a Novel Class of Nucleoside Analogs Effective Against HIV–1," *International Conference on AIDS,* Montreal, Quebec, Canada, Jun. 4–9, 1989.

Borthwick, et al., "Synthesis and Enzymatic Resolution of Carbocyclic 2'–Ara–Fluoro–Guanosine: A Potent New Anti–Herpetic Agent," *J. Chem. Soc. Commun.,* vol. 10. pp. 656–658 (1988).

Carter, et al., "Activities of (–)–Carbovir and 3'–Azido–3'–Deoxythymidine Against Human Immunodeficiency Virus In Vitro," *Antimicrobial Agents and Chemotherapy,* 34(6): 1297–1300 (1990).

Chang, Chien–Neng, et al., "Deoxycytidine Deaminase–resistant Steroisomer Is the Active Form of (±)–2', 3'–Dideoxy–3'–thiacytidine in the Inhibition of Hepatitis B Virus Replication," *The Journal of Biological Chemistry,* 267(20):13938–13942 (1992).

Chu, C.K., et al., "An Efficient Total Synthesis of 3'–Azido–3'–Deoxythiymidine (AZT) and 3'–Azido–2', 3'–Dideoxyuridine (AZDDU,CS–87) from D–Mannitol," *Tetrahedron Lett.,* 29(42):5349–5352 (1988).

Chu, et al., "Comparative Activity of 2',3'–Saturated and Unsaturated Pyrimidine and Purine Nucleosides Against Human Immunodeficiency Virus Type 1 in Peripheral Blood Mononuclear Cells," *Biochem. Pharm.,* 37(19):3543–3548 (1988).

Chu, et al., "Structure–Activity Relationships of Pyrimidine Nucleosides as Antiviral Agents for Human Immunodeficiency Virus Type 1 in Peripheral Blood Mononuclear Cells," *J. Med. Chem.,* 32:612 (1989).

Condreay, et al., "Evaluation of the Potent Anti–Hepatitis B Virus Agent (–) cis–5–Fluoro–1–[2–(Hydroxymethyl)–1, 3–Oxathiolan–5–yl]Cytosine in a Novel In Vivo Model," *Antimicrobial Agents and Chemotherapy,* 616–619 (1992).

Connolly and Hammer, "Minireview: Antiretroviral Therapy:Reverse Transcriptase Inhibition," *Antimicrobial Agents and Chemotherapy,* 36(2):245–254 (1992).

Cretton, E., et al., "Catabolism of 3'–Azido–3'–Deoxythymidine in Heptaocytes and Liver Microsomes, with Evidence of Formation of 3'–Amino–3'–Deoxythymidine, a Highly Toxic Catabolite for Human Bone Marrow Cells," *Moelcuilar Pharmacology,* 39:258–266 (1991).

Cretton, E., et al., "Pharmokinetics of 3'–Azido–3'–Dexoythymidine and its Catabolites and Interactions with Probenecid in Rhesus Monkeys," *Antimicrobial Agents and Chemotherapy,* 35(5):801–807 (1991).

Doong, Shin–Lian., et al., "Inhibition of the Replication of Hepatitis B Virus in vitro by 2',3'–Dideoxy–3'–Thiacytidine and Related Analogues," *Natl. Acad, Sci. USA,* 88:8495–8499 (1991).

Feorino, et al., "Prevention of activation of HIV–1 antiviral agents in OM–10.1 cells," *Antiviral Chem. & Chemotherapy,* 4(1):55–63 (1993).

Frick, et al., "Pharmacokinetics, Oral Bioavailability, and Metabolic Disposition in Rats of (–)–cis–5–Fluoro–1–[2–(Hydroxymethyl)–1,3–Oxathiolan–5–yl] Cytosine, a Nucleoside Analog Active against Human Immunodeficiency Virus and Hepatitis B Virus," *Antimicrobial Agents and Chemotherapy,* 37(11):2285–2292 (1993).

Furman, et al., "The Anti–Hepatitis B Virus Activities, Cytotoxicities, and Anabolic Profiles of the (–) and (+) Enantiomers of cis–5–Fluoro–1–[2(Hydromethyl)–1, 3–Oxthiolane–5–yl]Cytosine," *Antimicrobial Agents and Chemotherapy,* 36(12):2686–2692 (1992).

Herdewijn, et al., "Resolution of Aristeromycin Enantiomers," *J. Med. Chem.,* 1985, vol. 28, 1385–1386.

Hoong, et al., "Enzyme–Mediated Enantioselective Preparation of Pure Enantiomers of the Antiviral Agent 2',3'–Dideoxy–5–fluoro–3'–thiacytidine (FTC) and Related Compounds," *J. Org. Chem.,* 57:5563–5565 (1992).

Ito, et al., "Chirally Selective Synthesis of Sugar Moiety of Nucleosides by Chemicoenzymatic Approach: L–and D–Riboses, Showdomycin, and Cordycepin," *J. Am. Chem. Soc.,* 103:6739–6741 (1981).

Jansen, et al., "High–Capacity In Vitro Assessment of Anti–Hepatitis B Virus Compound Selectivity by a Virion–Specific Polymerase Chain Reaction Assay," *Antimicrobial Agents and Chemotherapy,* 441–447 (1993).

Jeong, L., et al., "Asymmetric Synthesis and Biological Evaluation of β–L–(2R,5S)–and a–L–(2R–5R)–1,3–Oxathiolane–Pyrimidine and –Purine Nucleosides and Potential Anti–HIV Agents," *J. Med. Chem.,* 36(2):181–195 (1993).

Krenitsky, T.A., et al., "3'–Amino–2',3'–Dideoxyribonucleosides of Some Pyrimidines: Synthesis and Biological Activities," *J. Med. Chem.,* vol. 26 (1983).

Krenitsky, et al., "Enzymic Synthesis of Purine D–arabinonucleosides," *Carbohydrate Research,* 97:139–146 (1981).

Lin, et al., "Potent and Selective In Vitro Activity of 3'–Deoxythmindine–2–Ene–(3'–Deoxy–2',3'Didehyrothymidine Against Human Immunodeficiency Virus," *Biochem. Pharm.,* 36(17):2713–2718 (1987).

Mahnoudian, et al., "Enzymatic Production of Optically Pure (2'R–cis)–2'–deoxy–3'–thiacytidine (3TC, Lamivudine): A Potent Anyi–HIV Agent," *Enzyme Microb. Technol.,* Sep. 1993, vol. 15, 749–755, published by the Glaxo Group Research.

Mitsuya, H., et al., 3'–Azido–3'–Deoxythmidine (BW A 509U): An Antiviral Agent that Inhibits the Infectivity and Cytopathic effect of Human T–Lymphotropic Virus Type III/Lymphadenopathy–Associated Virus *In Vitro, Proc. Natl. Acad. Sci., USA,* 82:7096–7100 (1985).

Mitsuya, H., et al., "Molecular Targets for AIDS Therapy," *Science,* vol. 249, pp. 1533–1544 (1990).

Mitsuya, H., et al., "Rapid in Vitro Systems for Assessing Activty of Agents Against HTLV–III/LAV," *AIDS:Modern Concepts and Therapeutic Challenges,* S. Broder, Ed. pp. 303–333, Marcel–Dekker, New York (1987).

Norbeck, D., et al., "A New 2',3'–Dideoxynucleoside Prototype with In Vitro Activity Against HIV," *Tetrahedron Lett.,* 30(46):6263–6266 (1989).

Ohno, et al., "Synthetic Studies on Biologically Active Natural Products by a Chemicoenzymatic Approach," *Tet. Letters,* 40:145–152 (1984).

Okabe, M., et al., "Synthesis of the Dideoxynucleosides ddC and CNT from Glutamic Acid, Ribonolactone, and Pyrimidine Bases," *J. Org. Chem.,* 53(20):4780–4786 (1988).

Paff, et al., "Intracellular Metabolism of (–)–and (+)–cis–5–Fluoro–1–[2–Hydroxymethy)–1, 3–Oxathiolan–5–yl]Cytosine in HepG2 Derivative 2.2.15 (Subclone P5Λ)Cells," *Antimicrobial Agents and Chemotherapy,* 1230–1238 (1994).

Pirkle and Pochansky, "Chiral Stationary Phases for the Direct LC Separation of Enantiomers," *Advances in Chromatography,* Giddings, J.C., Grushka, E., Brown, P.R., eds.: Marcel Dekker: New York, 1987: vol. 27, Chap. 3, pp. 73–127.

Richman, D.D., et al., "The Toxicity of Azidothymidine (AZT) in the Treatment of Patients with AIDS and AIDS–Related Complex," *N. Eng. J. Med.,* 317(4):192–197 (1987).

Roberts, et al., "Enzymic Resolution of cis–and trans–4–hydroxycyclopent–2–enylmethanol . . . " *J. Chem.. Soc.,* Perkin Trans. 1, (10):2605–7 (1991).

Saari, et al., "Synthesis and Evaluation of 2–Pyridinone Derivatives as HIV–1Specific Reverse Transcriptase Inhibitors, 2. Analogues of 3–Aminopyridin–2(1H)–one," *J. Med. Chem.,* 35:3792–3802 (1992).

Satsumabayashi, S. et al., "The Synthesis of 1,3–Oxathiolane–5–one Derivatives," *Bull, Chem. Soc. Japan,* 45:913–915 (1972).

Saunders, "Non–Nucleoside Inhibitors of HIV Reverse Transcriptase: Screening Successes–Clinical Failures," *Drug Design and Discovery,* 8:255–263 (1992).

Schinazi, R.F., et al., "Activities of the Four Optical Isomers of 2',3'–Dideoxy–3'–Thiacytidine (BCH–189) against Human Immunodeficiency Virus Type 1 in Human Lymphocytes," *Antimicrobial Agents and Chemotherapy* 36(3):672–676 (1992).

Schinazi, R.F., et al., "Insights into HIV Chemotherapy," *AIDS Research and Human Retroviruses* 8(6):963–990 (1992).

Schinazi, R.F., et al., "Pharmacokinetics and Metabolism of Racemic 2',3'–Dideoxy–5–Fluoro–3'–Thiacytidine in Rhesus Monkeys," *Antimicrobial Agents and Chemotherapy* 36(11):2432–2438 (1992).

Schinazi, R.F., et al., "Selective Inhibition of Human Immunodeficiency Viruses by Racemates and Enantiomers of cis–5–Fluoro–1–[2–(Hydroxymethyl)–1, 3–Oxathiolan–5–yl]Cytosine," *Antimicrobial Agents and Chemotherapy* 36(11):2433–2431 (1992).

Schinazi, R.F., et al., "Substrate Specificity of *Escherichia Coli* Thymidine Phosphorylase for Pyrimidine Nucleoside with an Anti–Human Immunodeficiency Virus Activity," *Biochemical Pharmacology* 44(2):199–204 (1992).

Secrist, et al., "Resolution of Racemic Carbocyclic Analogues of Purine Nucleosides Through the Action of Adenosine Deaminase Antiviral of the Carbocyclic 2'–Deoxyguanosine Enantiomers," *J. Med. Chem.,* vol. 30, pp. 746–749 (1987).

Shewach, et al., "Affinity of the antiviral enantiomers of oxathiolane cytosine nucleosides for human 2'–deoxycytidine kinase," *Biochem. Pharmacol.,* 45(7):1540–1543 (1993).

Sterzycki, R.Z., et al. "Synthesis and anti–HIV activity of several 2'–fluoro–containing pyrimidine nucleosides," *J. Med. Chem.,* 33(8):2150–2157 (1990).

Storer, R., et al., "The Resolution and Absolute Stereochemistry of the Enantiomeris of cis–1–2–(Hydromethyl)–1, 3–Oxathiolan–5–yl)cytosine (BCH189): Equipotent Anti–HIV Agents," *Nucleoside & Nucleotides,* 12(2):225–236 (1993).

van Roey, et al., "Solid State Conformation of Anti–Human Immnosudeficiency Virus Type–1 Agents: Crystal Structures of Three 3'–Azido–3'–deoxythymidine Analogues," *J. Am. Chem. Soc.,* 110:2277–2782 (1988).

Vorbrüggen, et al, "Nucleoside Synthesis with Trimethylsilyl Triflate and Perchlorate as Catalysts," *Chem. Ber.,* 114:1234–1255 (1981).

Wilson, et al., "The 5'–Triphosphates of the (1) and (–) Enantiomers of cis–5–Fluoro–1–[2–(Hydroxymethyl)–1, 3–Oxathiolane–5–yl]Cytosine Equally Inhibit Human Immunodeficiency Virus Type 1 Reverse Transcriptase," *Antimicrob. Agents and Chemother.,* 37(8):1720–1722 (1993).

Wilson, L.J., et al., "A General Method for Controlling Glycosylation Stereochemistry in the Synthesis of 2'–Deoxyribose Nucleosides," *Tetrahedron Lett.,* 31(13):1815–1818 (1990).

Wilson, L.J., et al., "The Synthesis and Anti–HIV Activity of Pyrimidine Dioxolanyl Nucleosides," *Bioorganic & Medicinal Chemistry Letters,* 3(2):169–174 (1993).

Winslow, et al., "In Vitro susceptibility of clinical isolates of HIV–1 to XM323, a non–peptidyl HIV protease inhibitor," *AIDS,* 8:753–756 (1994).

Zhu, Zhou, et al., "Cellular Metabolism of 3'–Azido, 3'–Dideoxyuridine with Formation of 5'–O–Diphophoshexase Derivatives by Previously Unrecognized Metabolic Pathways of 2'–Deoxyuridine Analogs," *Molecular Pharmacology,* 0:929–938 (1990).

FIGURE 1

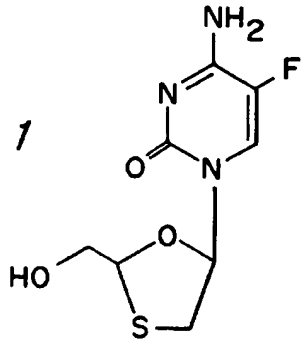


FIGURE 2

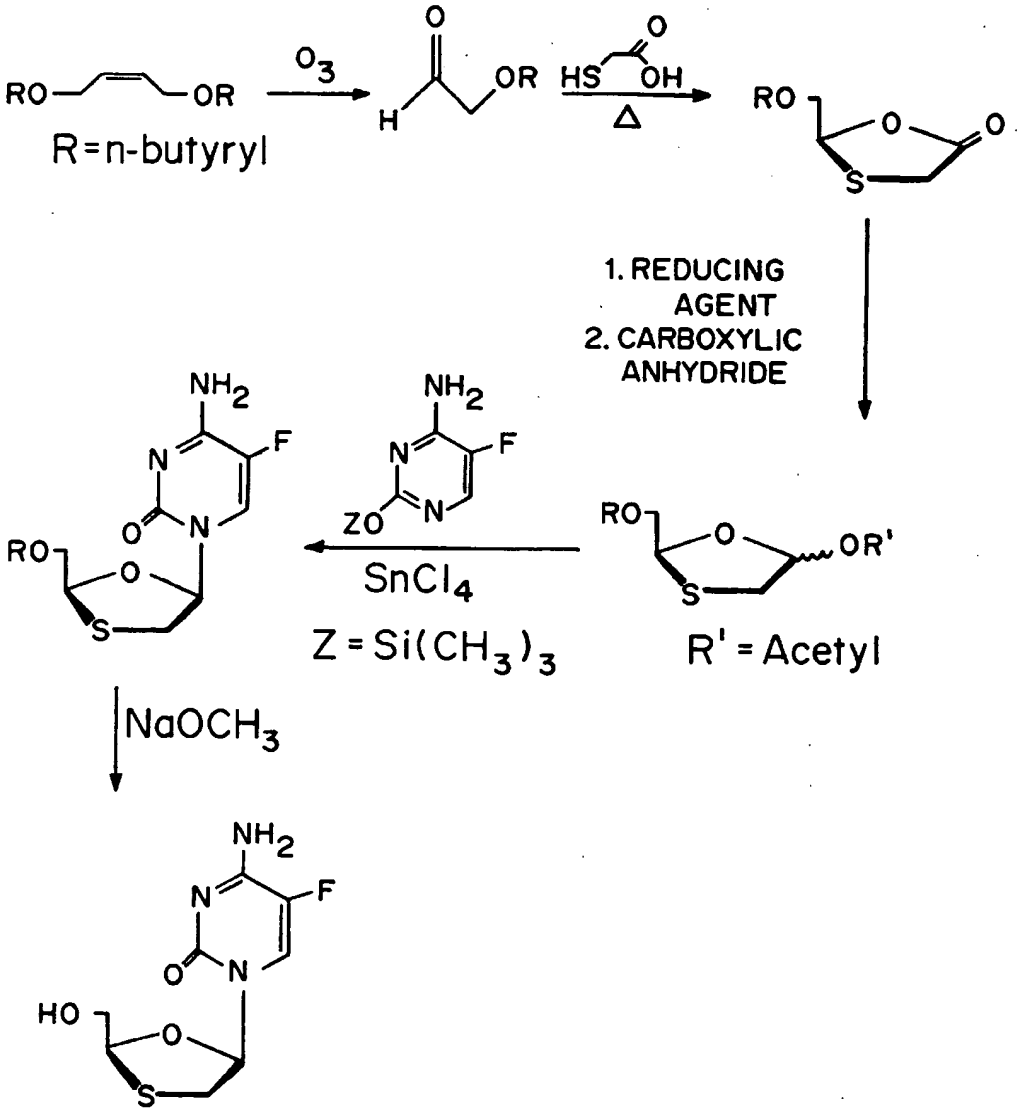


FIGURE 3

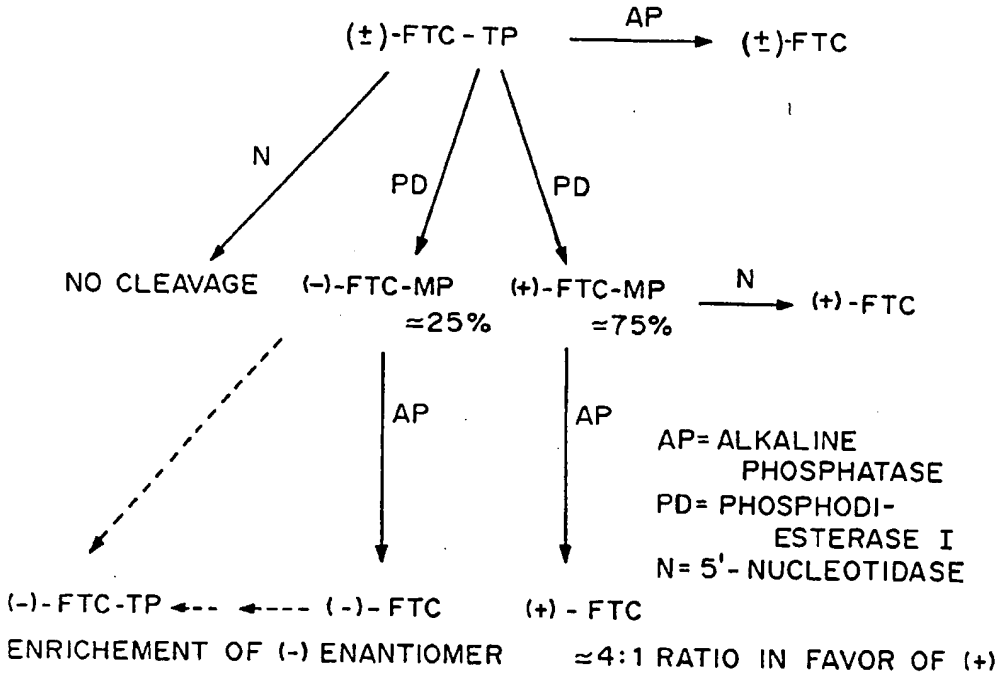


FIGURE 4

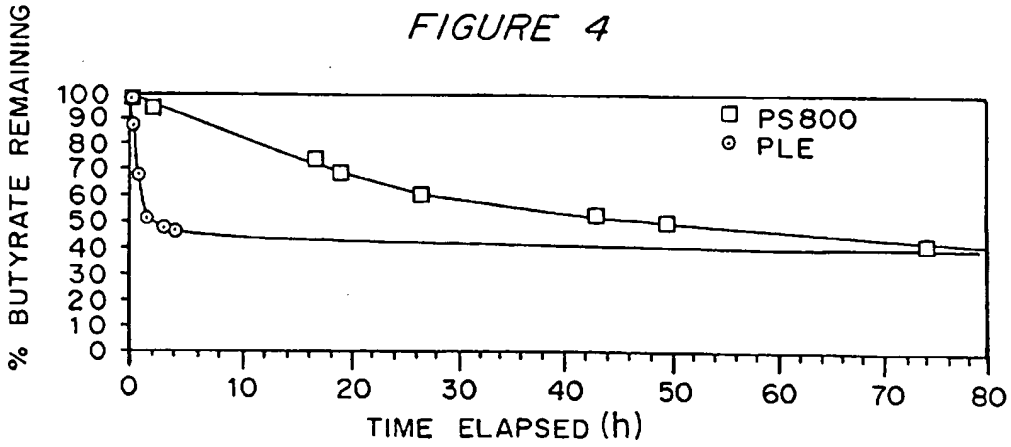


FIGURE 5

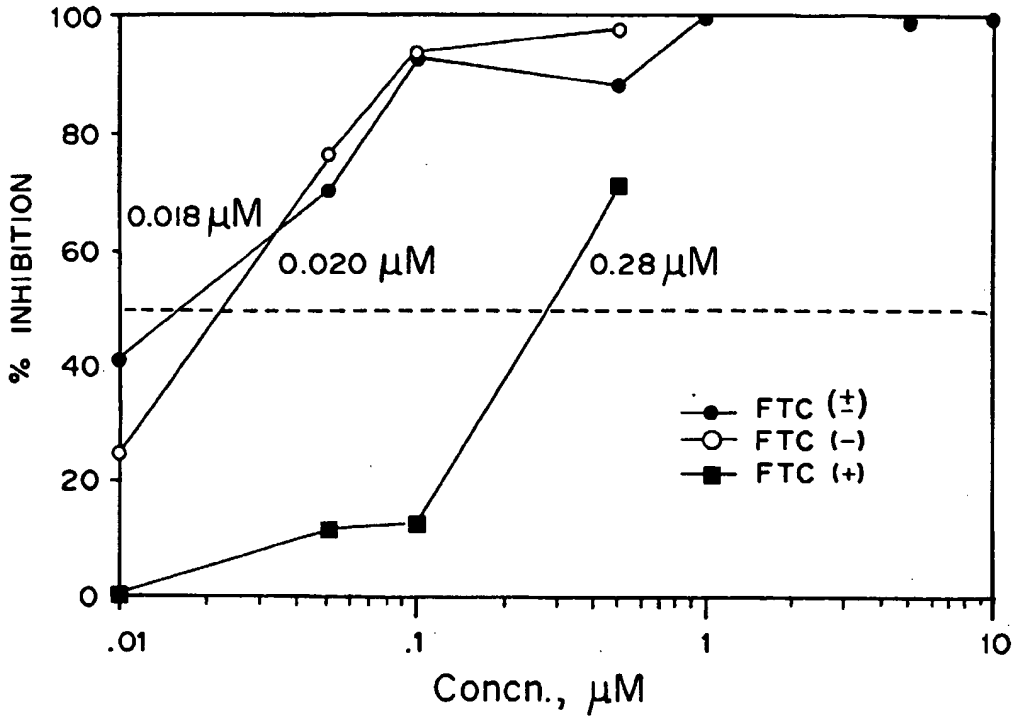
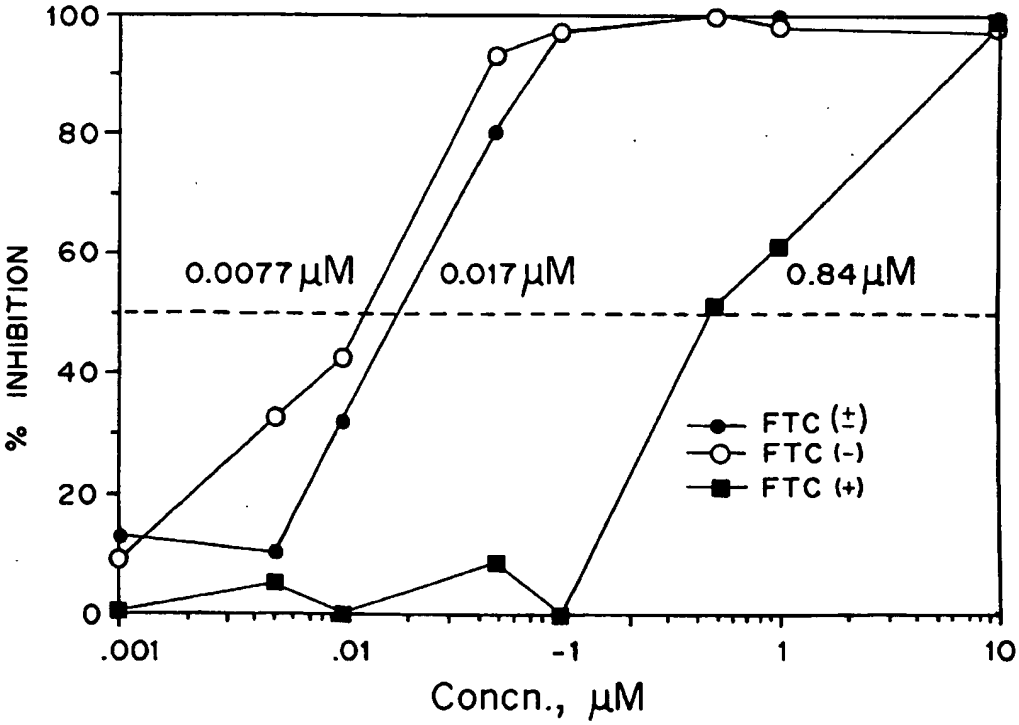


FIGURE 6



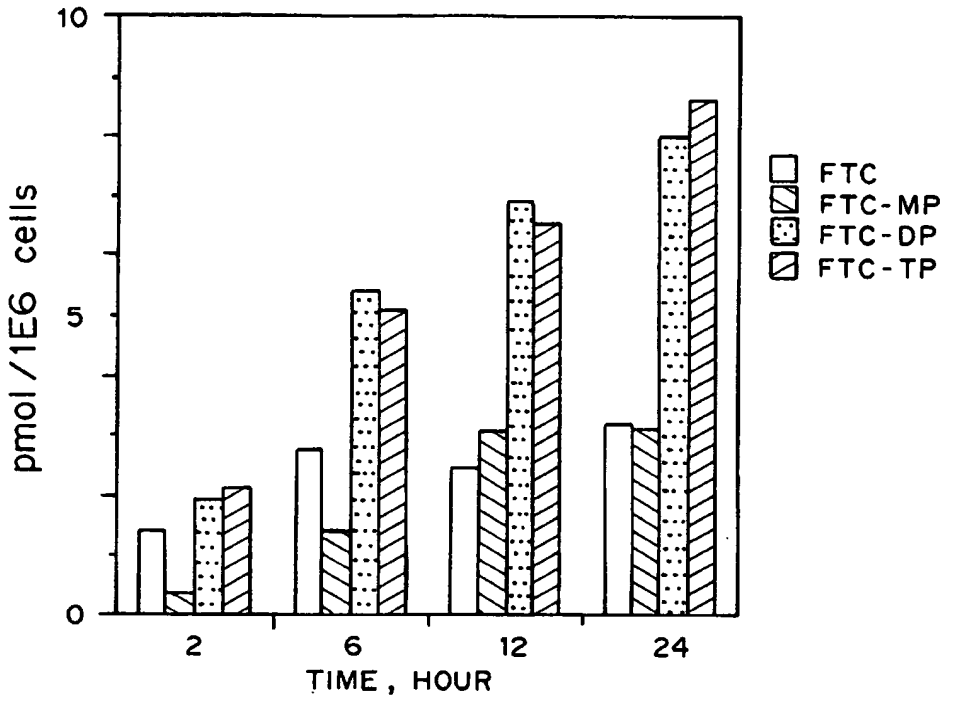


FIGURE 7

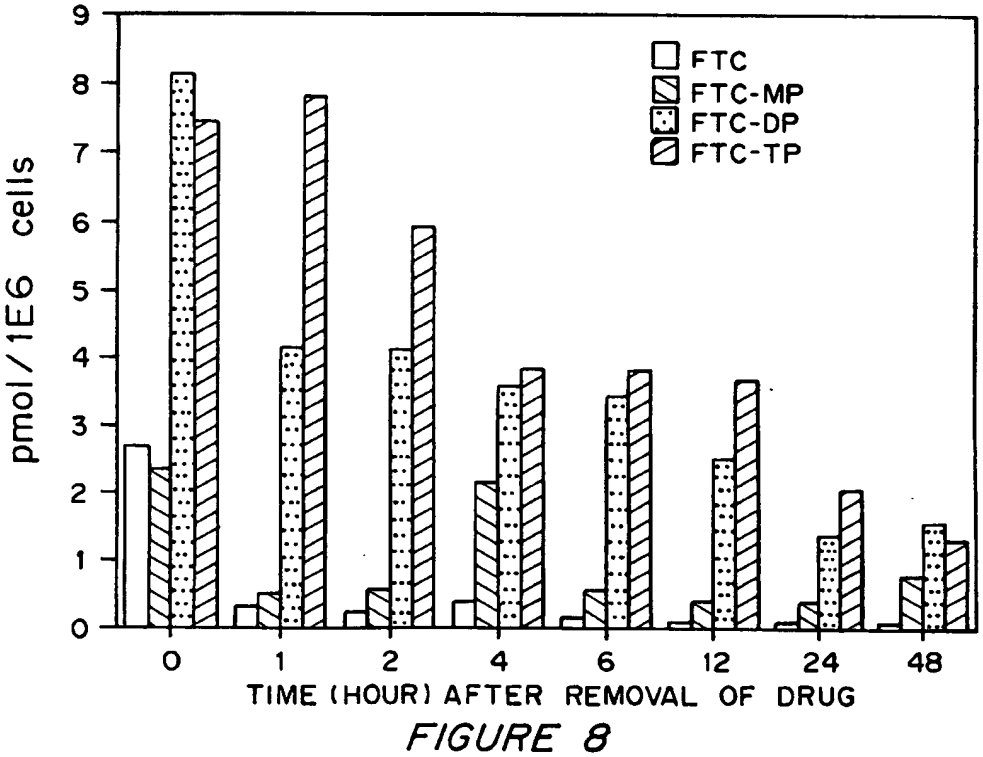


FIGURE 8

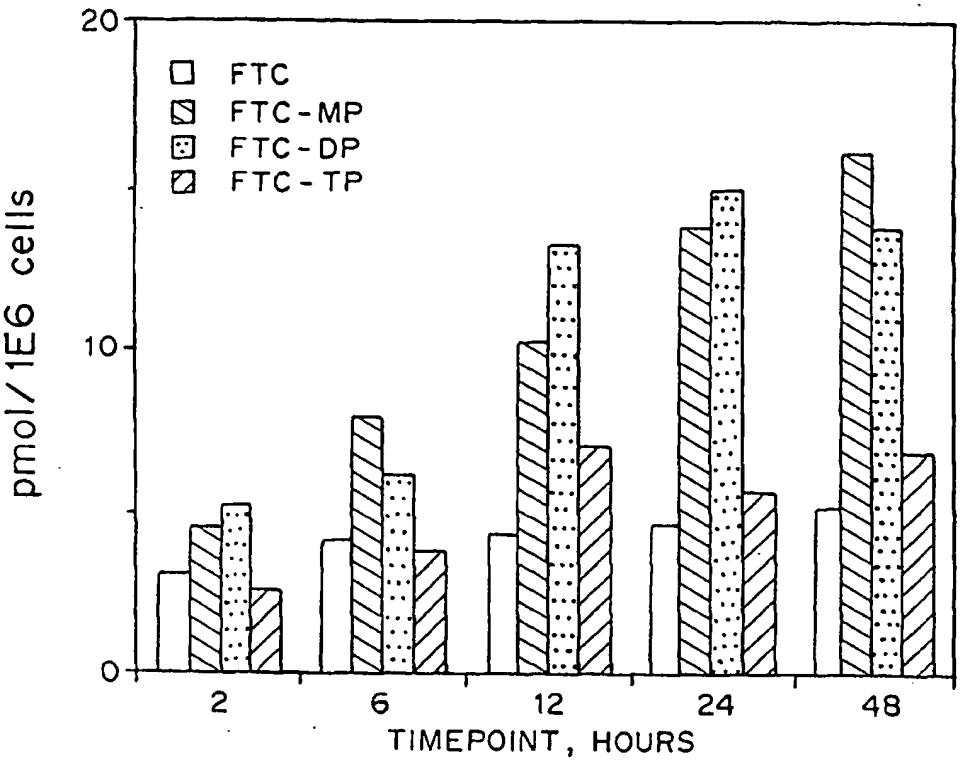


FIGURE 9

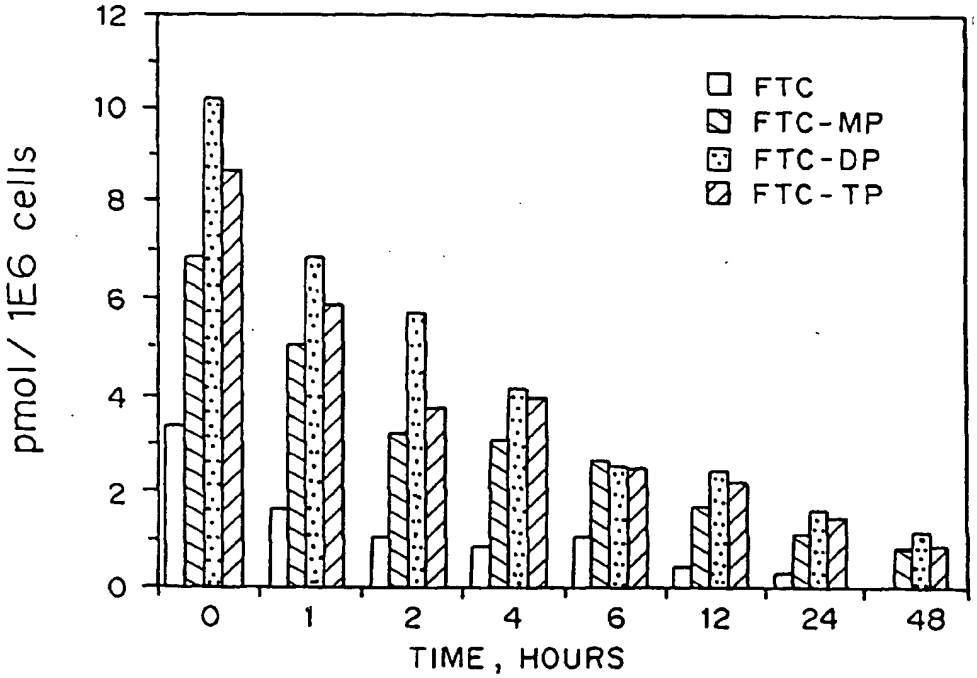


FIGURE 10

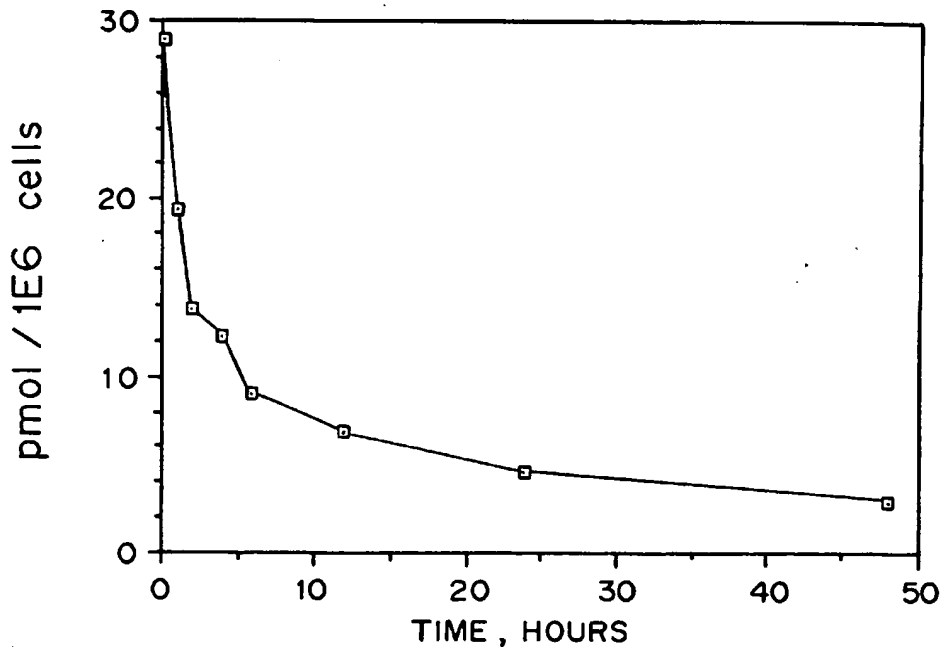


FIGURE 11

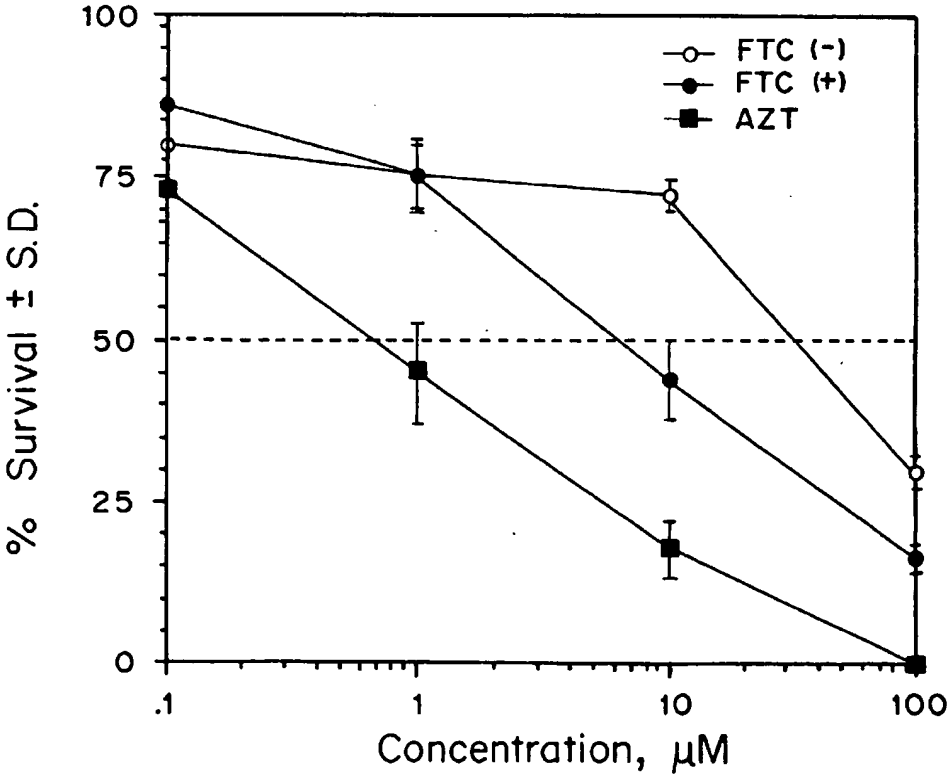


FIGURE 12

ANTIVIRAL ACTIVITY AND RESOLUTION OF 2-HYDROXYMETHYL-5-(5-FLUOROCYTOSIN-1-YL)-1,3-OXATHIOLANE

This application is a Continuation application of U.S. Ser. No. 07/831,153, filed on Feb. 12, 1992, by Dennis C. Liotta, Raymond F. Schinazi, and Woo-Baei Choi for "Antiviral Activity and Resolution of 2-hydroxymethyl-5(5-Fluorocytosin-1-yl)-1,3-Oxathiolane" which is a continuation-in-part application of (1) U.S. Ser. No. 07/659,760, now U.S. Pat. No. 5,210,085 entitled "Method for the Synthesis, Compositions and Use of 2'-Deoxy-5-Fluoro-3'-Thiacytidine and Related Compounds", filed on Feb. 22, 1991, by Dennis C. Liotta, Raymond F. Schinazi, and Woo-Baeg Choi, which is a continuation in part application of U.S. Ser. No. 07/473,318, now U.S. Pat. No. 5,204,466 entitled "Method and Compositions for the Synthesis of BCH-189 and Related Compounds", filed on Feb. 1, 1990, by Dennis C. Liotta and Woo-Baeg Choi and, (2) a continuation-in-part of U.S. Ser. No. 07/736,089, now abandoned, entitled "Method of Resolution and Antiviral Activity of 1,3-Oxathiolane Nucleoside Enantiomers" filed on Jul. 26, 1991, by Dennis C. Liotta, Raymond F. Schinazi, and Woo-Baeg Choi, and is a continuation-in-part of U.S. Ser. No. 07/659,760, now U.S. Pat. No. 5,210,085 filed on Feb. 22, 1991 referenced above.

The U.S. Government has rights in this invention arising out of the partial funding of work leading to this invention through the National Institutes of Health Grant Nos. AI-26055, AI-28731, NIH 5-21935, as well as a Veteran's Administration Merit Review Award.

BACKGROUND OF THE INVENTION

This invention is in the area of biologically active nucleosides, and specifically includes antiviral compositions that include 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane ("FTC"), its physiologically acceptable derivative, or physiologically acceptable salt, and a method for the resolution and use of the (−)-β-L and (+)-β-D enantiomers of FTC.

In 1981, acquired immune deficiency syndrome (AIDS) was identified as a disease that severely compromises the human immune system, and that almost without exception leads to death. In 1983, the etiological cause of AIDS was determined to be the human immunodeficiency virus (HIV). By December of 1990, the World Health Organization estimated that between 8 and 10 million people worldwide were infected with HIV, and of that number, between 1,000,000 and 1,400,000 were in the U.S.

In 1985, it was reported that the synthetic nucleoside 3'-azido-3'-deoxythymidine (AZT) inhibits the replication of human immunodeficiency virus. Since then, a number of other synthetic nucleosides, including 2',3'-dideoxyinosine (DDI), 2',3'-dideoxycytidine (DDC), 3'-fluoro-3'-deoxythymidine (FLT), and 2',3'-dideoxy-2',3'-didehydrothymidine (D4T), have been proven to be effective against HIV. A number of other 2',3'-dideoxynucleosides have been demonstrated to inhibit the growth of a variety of viruses in vitro. It appears that, after cellular phosphorylation to the 5'-triphosphate by cellular kinases, these synthetic nucleosides are incorporated into a growing strand of viral DNA, causing chain termination due to the absence of the 3'-hydroxyl group.

The success of various 2',3'-dideoxynucleosides in inhibiting the replication of HIV in vivo or in vitro has led a number of researchers to design and test nucleosides that

substitute a heteroatom for the carbon atom at the 3'-position of the nucleoside. Norbeck, et al., disclose that (±)-1-[(2β, 4β)-2-(hydroxymethyl)-4-dioxolanyl]thymine (referred to as (±)-dioxolane-T) exhibits a modest activity against HIV (EC_{50} of 20 μm in ATH8 cells), and is not toxic to uninfected control cells at a concentration of 200 μM. *Tetrahedron Letters* 30 (46), 6246, (1989). European Patent Application Publication No. 0 337 713 and U.S. Pat. No. 5,041,449, assigned to IAF BioChem International, Inc., disclose 2-substituted-4-substituted-1,3-dioxolanes that exhibit antiviral activity.

U.S. Pat. No. 5,047,407 and European Patent Application Publication No. 0 382 526, also assigned to IAF Biochem International, Inc. disclose a number of 2-substituted-5-substituted-1,3-oxathiolane nucleosides with antiviral activity, and specifically report that the racemic mixture (about the C4'-position) of the C1'-β isomer of 2-hydroxymethyl-5-(cytosin-1-yl)-1,3-oxathiolane (referred to below as (±)-BCH-189) has approximately the same activity against HIV as AZT, and no cellular toxicity at the tested levels. (±)-BCH-189 has also been found to inhibit the replication of AZT-resistant HIV isolates in vitro from patients who have been treated with AZT for longer than 36 weeks.

Another virus that causes a serious human health problem is the hepatitis B virus (referred to below as "HBV"). HBV is second only to tobacco as a cause of human cancer. The mechanism by which HBV induces cancer is unknown, although it is postulated that it may directly trigger tumor development, or indirectly trigger tumor development through chronic inflammation, cirrhosis, and cell regeneration associated with the infection.

After a two to six month incubation period in which the host is unaware of the infection, HBV infection can lead to acute hepatitis and liver damage, that causes abdominal pain, jaundice, and elevated blood levels of certain enzymes. HBV can cause fulminant hepatitis, a rapidly progressive, often fatal form of the disease in which massive sections of the liver are destroyed.

Patients typically recover from acute hepatitis. In some patients, however, high levels of viral antigen persist in the blood for an extended, or indefinite, period, causing a chronic infection. Chronic infections can lead to chronic persistent hepatitis. Patients infected with chronic persistent HBV are most common in developing countries. By mid-1991, there were approximately 225 million chronic carriers of HBV in Asia alone, and worldwide, almost 300 million carriers. Chronic persistent hepatitis can cause fatigue, cirrhosis of the liver, and hepatocellular carcinoma, a primary liver cancer.

In western industrialized countries, high risk groups for HBV infection include those in contact with HBV carriers or their blood samples. The epidemiology of HBV is very similar to that of acquired immune deficiency syndrome, which accounts for why HBV infection is common among patients with AIDS or AIDS-related complex. However, HBV is more contagious than HIV.

A human serum-derived vaccine has been developed to immunize patients against HBV. While it has been found effective, production of the vaccine is troublesome because the supply of human serum from chronic carriers is limited, and the purification procedure is long and expensive. Further, each batch of vaccine prepared from different serum must be tested in chimpanzees to ensure safety. Vaccines have also been produced through genetic engineering. Daily treatments with α-interferon, a genetically engineered

protein, has also shown promise. However, to date there is no known pharmaceutical agent that effectively inhibits the replication of the virus.

To market a nucleoside for pharmaceutical purposes, it must not only be efficacious with low toxicity, it must also be cost effective to manufacture. An extensive amount of research and development has been directed toward new, low cost processes for large scale nucleoside production. 2',3'-Dideoxynucleosides are currently prepared by either of two routes: derivatization of an intact nucleoside or condensation of a derivatized sugar moiety with a heterocyclic base. Although there are numerous disadvantages associated with obtaining new nucleoside analogues by modifying intact nucleosides, a major advantage of this approach is that the appropriate absolute stereochemistry has already been set by nature. However, this approach cannot be used in the production of nucleosides that contain either nonnaturally occurring bases or nonnaturally occurring carbohydrate moieties (and which therefore are not prepared from intact nucleosides), such as 1,3-oxathiolane nucleosides and 1,3-dioxolane nucleosides.

When condensing a carbohydrate or carbohydrate-like moiety with a heterocyclic base to form a synthetic nucleoside, a nucleoside is produced that has two chiral centers (at the C1' and C4'-positions), and thus exists as a diastereomeric pair. Each diastereomer exists as a set of enantiomers. Therefore, the product is a mixture of four enantiomers.

It is often found that nucleosides with nonnaturally-occurring stereochemistry in either the C1' or the C4'-positions are less active than the same nucleoside with the stereochemistry as set by nature. For example, Carter, et al., have reported that the concentration of the (−)-enantiomer of carbovir (2',3'-didehydro-2',3'-dideoxyguanosine) in cell culture required to reduce the reverse transcriptase activity by 50% (EC_{50}) is 0.8 μM, whereas the EC_{50} for the (+)-enantiomer of carbovir is greater than 60 μM. *Antimicrobial Agents and Chemotherapy*, 34:6, 1297–1300 (June 1990).

PCT International Publication No. WO 91/11186 discloses that 1,3-oxathiolane nucleosides can be prepared with high diastereoselectivity (high percentage of nucleoside with a β configuration of the bond from the C1'-carbon to the heterocyclic base) by careful selection of the Lewis acid used in the condensation process. It was discovered that condensation of a 1,3-oxathiolane nucleoside with a base occurs with almost complete β-stereospecificity when stannic chloride is used as the condensation catalyst. Other Lewis acids provide low (or no) C1'-β selectivity or simply fail to catalyze the reactions.

In light of the fact that acquired immune deficiency syndrome, AIDS-related complex, and hepatitis B virus have reached epidemic levels worldwide, and have tragic effects on the infected patient, there remains a strong need to provide new effective pharmaceutical agents to treat these diseases that have low toxicity to the host.

There is also a need to provide a cost effective, commercially viable method to produce pharmaceutically important nucleosides, and specifically attain β-stereospecificity in the C4'-position of synthetic nucleosides prepared by condensing a carbohydrate-like moiety with a base.

Therefore, it is an object of the present invention to provide a method and composition for the treatment of human patients infected with HIV.

It is another object of the present invention to provide a method and composition for the treatment of human patients or other host animals infected with HBV.

It is still another object of the present invention to provide enantiomerically enriched 1,3-oxathiolane nucleosides.

It is still another object of the present invention to provide a method for the resolution of C4'-enantiomers of 1,3-oxathiolane nucleosides.

SUMMARY OF THE INVENTION

A method and composition for the treatment of HIV and HBV infections in humans and other host animals is disclosed that includes administering an effective amount of 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane, a pharmaceutically acceptable derivative thereof, including a 5' or N^4 alkylated or acylated derivative, or a pharmaceutically acceptable salt thereof, in a pharmaceutically acceptable carrier.

It has been discovered that 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane ("FTC"), exhibits surprisingly high activity against human immunodeficiency virus with very low host cell toxicity. It has also been discovered that FTC exhibits very significant activity against HBV, and therefore can be used to treat patients who have a variety of illnesses associated with HBV infection.

Toxicity and pharmacokinetic studies confirm the usefulness of FTC as an antiviral agent for pharmaceutical administration. FTC and its enantiomers are nontoxic to peripheral human bone marrow cells at concentrations up to 50 μM and other cell lines at concentrations up to 200 μM. FTC-TP is a major intracellular metabolite in PBMC and HepG2 cells. FTC-TP competitively inhibits HIV-1 reverse transcriptase (RT) with a K_I of 0.2 μM using a poly(I)oligo(dC) template-primer. Using sequencing analysis, FTC-TP can be shown to be a potent DNA chain terminator when HIV-RT is used (C-stops).

Chronic treatment with FTC is not toxic to rodents, even at oral doses of 85 mg/kg per day for at least two months. The pharmacokinetics of FTC in rhesus monkeys indicates high oral bioavailability (approximately 73±6%) and a plasma terminal half life of approximately 1.34±0.18 (mean of oral and I.V. administration).

A process for the resolution of a racemic mixture of nucleoside enantiomers, including the racemic mixture of FTC, is also disclosed that includes the step of exposing the racemic mixture to an enzyme that preferentially catalyzes a reaction in one of the enantiomers. The process can be used to resolve a wide variety of nucleosides, including pyrimidine and purine nucleosides that are optionally substituted in the carbohydrate moiety or base moiety. The process can also be used to resolve nucleoside derivatives that contain additional heteroatoms in the carbohydrate moiety, for example, (±)-FTC and (±)-BCH-189. The resolution of nucleosides can be performed on large scale at moderate cost.

Using methods described herein, FTC was resolved into its (+)-β-D and (−)-β-L enantiomers. The (+)-β-L-enantiomer appears to be more potent that the (+)-β-D-enantiomer against HIV, HBV, and SIV. The (+)-enantiomer of FTC is also active against HIV, HBV, and SIV.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 is an illustration of the chemical structure of 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane ("FTC").

FIG. 2 is an illustration of a method for the preparation of 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane.

FIG. 3 is a flow chart of the specificity of alkaline phosphatase and snake venom phosphodiesterase for the (+) and (−) enantiomers of FTC.

FIG. 4 is a graph indicating the progress of lipase-catalyzed hydrolysis of the 5'-butyryl ester of FTC over time using the enzymes Amano PS-800® (-open square-) and PLE (-open circle with dot-).

FIG. 5 is a graph of the effect of concentration (μM) of racemic and enantiomerically enriched FTC (prepared by the method of Example 4) versus the percent inhibition of human PBM cells infected with HIV-1. ((-darkened circle-, (±)-FTC), (-open circle-,(-)-FTC), (-darkened square-, (+)-FTC).

FIG. 6 is a graph of the effect of concentration (μM) of racemic and enantiomerically enriched FTC (prepared by method of Example 3) on the percent inhibition of human PBM cells infected with HIV-1. ((-darkened circle-, (+)-FTC), (-open circle-,(-)-FTC), (-darkened square-,(+)-FTC).

FIG. 7 is a graph of the uptake of tritiated (±)-FTC in human PBM cells (average of two determinations) in time (hours) versus pmol/10^6 cells.

FIG. 8 is a graph of the egress of radiolabeled (±)-FTC from human PBM cells, measured in hours versus pmol/10^6 cells.

FIG. 9 illustrates the presence of [^{3}H]-(±)-FTC and its phosphorylated derivatives in human HepG-2 cells (average of two determinations) incubated in media containing 10 μM [^{3}H]-(±)-FTC, measured in pmol/10^6 cells over time.

FIG. 10 illustrates the egress of [^{3}H]-(±)-FTC and its phosphorylated derivatives in human HepG2 in pmol/10^6 cells over time cells after pulsing cells with 10 μM [^{3}H]-(±)-FTC (700 DPM/pmole) for 24 hours, and evaluating the concentration of compound 24 hours after removal.

FIG. 11 illustrates the decrease in the combined concentration of [^{3}H]-(±)-FTC and its phosphorylated derivatives from human HepG2 cells after incubation with 10 μM [^{3}H]-(±)-FTC (700 DPM/pmole) for 24 hours, in pmol/10^6 cells over time.

FIG. 12 is a graph of the effect of the enantiomers of FTC on colony formation of granulocyte-macrophage precursor cells, as measured in percent survival versus concentration in μM ((-)-FTC, open circle; (+)-FTC, darkened circle; AZT, darkened square.

DETAILED DESCRIPTION OF THE INVENTION

As used herein, the term "enantiomerically enriched nucleoside" refers to a nucleoside composition that includes at least 95% of a single enantiomer of that nucleoside.

As used herein, the term FTC refers to 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane (the racemic form or enantiomers), also referred to as 2'-deoxy-5-fluoro-3'-thiacytidine.

As used herein, the term (±)-FTC refers to (±)-β-D,L-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane.

As used herein, the term (-)-FTC refers to (-)-β-L-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane.

As used herein, the term (+)-FTC refers to (+)-β-D-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane.

As used herein, the terms FTC-MP, FTC-DP, and FTC-TP refer to the monophosphate, diphosphate, and triphosphate of FTC, respectively.

As used herein, the term BCH-189 refers to 2-hydroxymethyl-5-(cytosin-1-yl)-1,3-oxathiolane.

As used herein, the term "preferential enzyme catalysis" refers to catalysis by an enzyme that favors one substrate over another.

As used herein, a leaving group means a functional group that forms an incipient carbonation when it separates from the molecule that it is attached to.

The invention as disclosed herein is a method and composition for the treatment of HIV and HBV infections, and other viruses replicating in like manner, in humans or other host animals, that includes administering an effective amount of the (±)-β-D,L, the (-)-β-L or (+)-β-D enantiomer of 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane, a pharmaceutically acceptable derivative, including a 5' or N^4 alkylated or acylated derivative, or a pharmaceutically acceptable salt thereof, in a pharmaceutically acceptable carrier. As shown below, the compounds of this invention either possess antiretroviral activity, such as anti-HIV-1, anti-HIV-2 and anti-simian immunodeficiency virus (anti-SIV) activity, themselves or are metabolized to a compound that exhibits antiretroviral activity.

FTC and its pharmaceutically acceptable derivatives or salts or pharmaceutically acceptable formulations containing these compounds are useful in the prevention and treatment of HIV infections and other related conditions such as AIDS-related complex (ARC), persistent generalized lymphadenopathy (PGL), AIDS-related neurological conditions, anti-HIV antibody positive and HIV-positive conditions, Kaposi's sarcoma, thrombocytopenia purpurea and opportunistic infections. In addition, these compounds or formulations can be used prophylactically to prevent or retard the progression of clinical illness in individuals who are anti-HIV antibody or HIV-antigen positive or who have been exposed to HIV.

FTC and its pharmaceutically acceptable derivatives or pharmaceutically acceptable formulations containing these compounds are also useful in the prevention and treatment of HBV infections and other related conditions such as anti-HBV antibody positive and HBV-positive conditions, chronic liver inflammation caused by HBV, cirrhosis, acute hepatitis, fulminant hepatitis, chronic persistant hepatitis, and fatigue. These compounds or formulations can also be used prophylactically to prevent or retard the progression of clinical illness in individuals who are anti-HBV antibody or HBV-antigen positive or who have been exposed to HBV.

FTC can be converted into a pharmaceutically acceptable ester by reaction with an appropriate esterifying agent, for example, an acid halide or anhydride. FTC or its pharmaceutically acceptable derivative can be converted into a pharmaceutically acceptable salt thereof in a conventional manner, for example, by treatment with an appropriate base. The ester or salt of FTC can be converted into FTC, for example, by hydrolysis.

In summary, the present invention includes the following features:

(a) (±)-β-D,L-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathioiane and pharmaceutically acceptable derivatives and salts thereof;.

(b) (-)-β-L-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane and pharmaceutically acceptable derivatives and salts thereof;

(c) (+)-β-D-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane and pharmaceutically acceptable derivatives and salts thereof;

(d) (±)-β-D,L-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane, its (-) and (+) enantiomers, and pharmaceutically

acceptable derivatives and salts thereof for use in medical therapy, for example for the treatment or prophylaxis of a HIV or HBV infection;

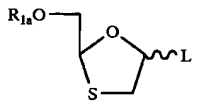
(e) use of (±)-β-D,L-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane, its (−) and (+) enantiomers, and pharmaceutically acceptable derivatives and salts thereof in the manufacture of a medicament for treatment of a HIV or HBV infection;

(f) pharmaceutical formulations comprising (+)-β-D,L-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane, its (−) or (+) enantiomer, or a pharmaceutically acceptable derivative or salt thereof together with a pharmaceutically acceptable carrier or diluent;

(g) a process for the preparation of 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane which comprises:

(i) reacting optionally protected 5-fluorocytosine with a 1,3-oxathiolane of formula A

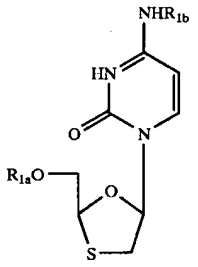
A



wherein R_{1a} is hydrogen or a hydroxyl protecting group, including an acyl group, and L is a leaving group; and optionally removing any hydroxyl protecting group.

(ii) reacting a compound of formula B

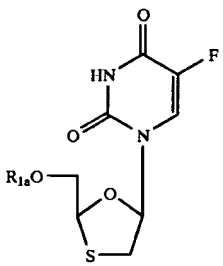
B



(wherein R_{1a} is as defined above and R_{1b} is an amino protecting group) with a fluorinating agent serving to introduce a fluorine atom in the 5-position of the cytosine ring; or

(iii) reacting a compound of formula C

C



(wherein R_{1a} is as defined above) with an agent serving to convert the oxo group in the 4-position of the uracil ring to an amino group; any remaining protecting groups being removed to produce the desired product.

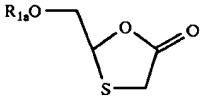
f) a process for the preparation of a (−) or (+) enantiomer of 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane which comprises subjecting the compound or derivative (e.g. 5'-ester) thereof in the form of a mixture of (−) and (+) enantiomers to conditions or reacting with reagents serving to separate the enantiomers and if necessary converting the resulting derivative to the parent compound.

With regard to process e) (i), the-hydroxy protecting group includes protecting groups described in detail below, including acyl (e.g. acetyl), arylacyl (e.g. benzoyl or substituted benzoyl), trityl or monomethoxytrityl, benzyl or substituted benzyl, trisubstituted silyl, including trialkylsilyl (e.g. dimethyl-t-butylsilyl) or diphenylmethylsilyl. The 5-fluorocytosine compound can be optionally protected with silyl, e.g., trisubstituted silyl groups. The protecting groups can be removed in a conventional manner. The leaving group L is a leaving group typical of those known in the art of nucleoside chemistry, e.g. halogen such as chlorine or bromine, alkoxy such as methoxy or ethoxy, or acyl such as acetyl or benzoyl.

The reaction in process e) (i) can be carried out in an organic solvent (e.g., 1,2-dichloroethane or acetonitrile) in the presence of a Lewis acid, preferably stannic chloride, or trimethylsilyl triflate.

Compounds of formula A (wherein L represents an acyl group, e.g., an acetyl group) can be obtained by reaction of a compound of formula D

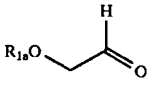
D



(wherein R_1 is defined above) with a reducing agent, e.g., a lithium aluminum hydride compound, following by treatment with the appropriate conventional reagent for the desired intermediate, for example, a carboxylic acid anhydride, e.g. acetic anhydride, for acylation, chlorinating or brominating reagents for halogenation, or alkylating reagents.

The compound of formula D can be prepared by reaction of a compound of formula E

E



with $HSCH_2CO_2H$ at an elevated temperature.

The compound of formula E can be prepared by ozonolysis of an allyl ether or ester having the formula CH_2=CH—CH_2—OR or a diether or diester of 2-butene-1,3-diol having the formula $ROCH_2$—CH=CH—CH_2OR, in which R is a protecting group, such as an alkyl, silyl, or acyl group.

With regard to process e) (ii), the 5-fluoro substituent can be introduced by methods known in the art (M. J. Robins, et al., in Nucleic Acid Chemistry, Part 2, L. B. Townsend and R. S. Tipson, editors,. J. Wiley and Sons, New York, 895–900 (19/8) and references therein; R. Duschinsky in Nucleic Acid Chemistry, Part 1, L. B. Townsend and R. S. Tipson, editors, J. Wiley and Sons, New York 43–46(1978) and references therein). The fluorinating agent may be, for example, trimethylhypofluorite in fluorotrichloromethane.

With regard to process e) iii), the compound of formula C can be treated with 1,2,4-triazole, together with

4-chlorophenyl dichlorophosphate, to form the corresponding 4-(1,2,4-triazoylyl) compound which is then converted to the desired 4-amino (cytidine) compound by reaction with for example methanol.

The starting materials of formulas B and C can be prepared for example by reaction of an appropriate (optionally protected) base with a compound of formula A in an analogous manner to that described in process e) i). 5-Fluorouracil and 5-fluorocytosine are commercially available from Aldrich Chemical Co., Milwaukee, Wis. 53233, USA.

Resolution of the (±)-enantiomers can be accomplished as specified in detail in Section III. below.

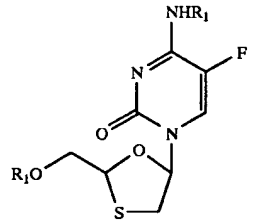
FTC can be converted into a pharmaceutically acceptable ester by reaction with an appropriate esterifying agent, for example, an acid halide or anhydride. FTC or its pharmaceutically acceptable derivative can be converted into a pharmaceutically acceptable salt thereof in a conventional manner, for example, by treatment with an appropriate base. The ester or salt of FTC can be converted into FTC, for example, by hydrolysis.

I. Active Compound, and Physiologically Acceptable Derivatives and Salts Thereof

The antivirally active compound disclosed herein is 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane (see FIG. 1), in the racemic form or as an isolated enantiomer.

The active compound can be administered as any derivative that upon administration to the recipient, is capable of providing directly or indirectly, the parent FTC compound, or that exhibits activity itself. Nonlimiting examples are the pharmaceutically acceptable salts (alternatively referred to as "physiologically acceptable salts"), and the 5' and N^4 acylated or alkylated derivatives of the active compound (alternatively referred to as "physiologically active derivatives"). In one embodiment, the acyl group is a carboxylic acid ester in which the non-carbonyl moiety of the ester group is selected from straight, branched, or cyclic alkyl, alkoxyalkyl including methoxymethyl, aralkyl including benzyl, aryloxyalkyl such as phenoxymethyl, aryl including phenyl optionally substituted with halogen, C_1 to C_4 alkyl or C_1 to C_4 alkoxy, sulfonate esters such as alkyl or aralkyl sulphonyl including methanesulfonyl, the mono, di or triphosphate ester, trityl or monomethoxytrityl, substituted benzyl, trialkylsilyl (e.g. dimethyl-t-butylsilyl) or diphenylmethylsilyl. Aryl groups in the esters optimally comprise a phenyl group. The alkyl group can be straight, branched, or cyclic, and is optimally a C_1 to C_{18} group.

Specific examples of pharmaceutically acceptable derivatives of FTC include, but are not limited to:



wherein R_1 and R_2 are independently selected from the group consisting of alkyl and acyl, specifically including but not limited to methyl, ethyl, propyl, butyl, pentyl, hexyl, isopropyl, isobutyl, sec-butyl, t-butyl, isopentyl, amyl, t-pentyl, 3-methylbutyryl, hydrogen succinate, 3-chlorobenzoate, cyclopentyl, cyclohexyl, benzoyl, acetyl, pivaloyl, mesylate, propionyl, butyryl, valeryl, caproic, caprylic, capric, lauric, myristic, palmitic, stearic, oleic, amino acids including but not limited to alanyl, valinyl, leucinyl, isoleucinyl, prolinyl, phenylalaninyl, tryptophanyl, methioninyl, glycinyl, serinyl, threoninyl, cysteinyl, tyrosinyl, asparaginyl, glutaminyl, aspartoyl, glutaoyl, lysinyl, argininyl, and histidinyl, and wherein one of R_1 and R_2 can be H.

FTC or its derivatives can be provided in the form of pharmaceutically acceptable salts. As used herein, the term pharmaceutically acceptable salts or complexes refers to salts or complexes of FTC that retain the desired biological activity of the parent compound and exhibit minimal, if any, undesired toxicological effects. Nonlimiting examples of such salts are (a) acid addition salts formed with inorganic acids (for example, hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid, and the like), and salts formed with organic acids such as acetic acid, oxalic acid, tartaric acid, succinic acid, malic acid, ascorbic acid, benzoic acid, tannic acid, pamoic acid, alginic acid, polyglutamic acid, naphthalenesulfonic acids, naphthalenedisulfonic acids, and polygalacturonic acid; (b) base addition salts formed with polyvalent metal cations such as zinc, calcium, bismuth, barium, magnesium, aluminum, copper, cobalt, nickel, cadmium, sodium, potassium, and the like, or with an organic cation formed from N,N-dibenzylethylenediamine, ammonium, or ethylenediamine; or (c) combinations of (a) and (b); e.g., a zinc tannate salt or the like.

Modifications of the active compound, specifically at the N^4 and 5'-0 positions, can affect the bioavailability and rate of metabolism of the active species, thus providing control over the delivery of the active species. Further, the modifications can affect the antiviral activity of the compound, in some cases increasing the activity over the parent compound. This can easily be assessed by preparing the derivative and testing its antiviral activity according to the methods described herein, or other method known to those skilled in the art.

II. Preparation of the Active Compounds

The racemic mixture of FTC can be prepared according to the method disclosed in detail in PCT International Publication No. WO 91/11186, published on Aug. 8, 1991, and filed by Emory University, or by the method disclosed in Example 1. In general, the method includes ozonizing either an allyl ether or ester having the formula CH_2=CH—CH_2—OR or a diether or diester of 2-butene-1,3-diol having the formula $ROCH_2$—CH=CH—CH_2OR, in which R is a protecting group, such as an alkyl, silyl, or acyl group, to form a glycoaldehyde having the formula OHC—CH_2—OR; adding thioglycolic acid to the glycoaldehyde to form a lactone of the formula 2-(R-oxy)-methyl-5-oxo-1,3-oxathiolane; reducing the lactone to various compounds containing a leaving group at the 5 position of the oxathiolane ring; coupling these compounds with silyated 5-fluorocytosine in the presence of $SnCl_4$ to form the β-isomer of FTC; and optionally removing the protecting groups.

EXAMPLE 1

Preparation of (±)-β-D,L-2-Hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane

A method for the preparation of the racemic mixture of FTC is illustrated in FIG. 2, and described in detail below.

Protection of 2-butene-1,4-diol

In a dry, 2 L, 3-neck flask under inert atmosphere, 100 grams (93.5 ml=1.135 mol=1.00 eq.) of 2-butene-1,4-diol and 15 grams (approx. 0.1 eq.) of DMAP

(4-dimethylaminopyridine) were dissolved in 800 ml of dry pyridine and stirred while cooling to 0° C. Butyryl chloride (260 ml=2.2 eq) was then added slowly to prevent overheating and allowed to stir for one hour. The reaction was quenched with a small amount of ice water. The liquid was decanted off from the salt and evaporated in vacuo. The remaining salt was dissolved in water and the aqueous solution was extracted twice with ethyl ether. The combined other layers were washed once with saturated $CuSO_4$, twice with saturated $NaHCO_3$ containing Norit®, and then vacuum filtered through a celite® plug.

The concentrated reaction mixture was dissolved in ether and washed following the same procedure as above for the salt solution. The combined organic layers were concentrated by rotary evaporation, then placed under vacuum. This reaction is typically very close to quantitative. The scale can be easily increased as necessary. The product, 1,4-dibutyryl-2-butene-1,4-diol is a colorless to slightly yellow, clear liquid.

Ozonolysis of the protected diol

1,4-Dibutyryl-2-butene-1,4-diol (1.365 mol) was dissolved in 4 L of dry CH_2Cl_2 in a dry, 5 L 3-neck flask equipped with a large drying tube and an open tube for the introduction of gas. The tube is optimally not a fritted, gas bubbling tube that will clog on exposure to the concentrated solution. The solution was stirred and cooled to −78° C. while inert gas was bubbled through the solution. The gas inlet was sealed once the solution had cooled sufficiently, and the flask and stirring apparatus were moved to the ozone generator. Oxygen was bubbled through the stirring solution for at least 20 minutes while maintaining the ice bath. A Cryocool is ideal to maintain the low temperature for this lengthy reaction. The ozone was then introduced at 8 to 8.5 psi. Upon completion, the ozone flow was stopped, and oxygen was bubbled through the solution for about a half an hour before 3 equivalents of Me_2S were added. The flask was removed from the cooling bath and transported to a hood where it was stirred for about 2 days to affect complete reduction. The solution was evaporated and put under vacuum for several hours.

This reaction typically yields approximately 95% of protected aldehyde (2-butyryloxyacetaldehyde), a colorless to yellow, clear liquid.

Cyclization of the Aldehyde with Mercaptoacetic acid

The aldehyde (1.0 equivalent) was dissolved in toluene to provide a 0.80 to 0.85M solution in a flask equipped with a Dean Stark-type trap. Thioglycolic acid (1.1 equiv.) was added and the mixture was heated to reflux. Water was azeotropically removed via the trap. The reaction was completed in 3 hours and was allowed to cool to room temperature. The organic solution was washed twice with equal volumes of sat. $NaHCO_3$ water and once with water, dried over $MgSO_4$ and Norit, and vacuum filtered through celite before being evaporated in vacuo. The first $NaHCO_3$ wash was back extracted once with ether; the ether was washed once with water, dried over $MgSO_4$ and Norit®, vacuum filtered through celite®, and evaporated along with the other organic material from the toluene solution. The combined material was placed under vacuum overnight.

The reaction typically provides a 90% yield of 2-(butyryloxy)-methyl-5-oxo-1,3-oxathiolane.

Reduction of Lactone and Conversion to the Acetate

2-Butyryloxy-methyl-5-oxo-1,3-oxathiolane (1.00 equivalent) was dissolved in dry THF to give a 0.23M solution in a dry, 3-neck flask equipped with a mechanical stirrer and maintained under an inert atmosphere. The solution was stirred and cooled to 0° C. before 1.1 equivalent of 1.0M $Li(t\text{-}BuO)_3AlH$ in THF was added via canula. The reduction was complete in approximately three hours, as indicated by TLC using 2:1 ether/hexane solvent system and an anisaldehyde stain.

Approximately 10 equivalents of freshly distilled Ac_2O were then added and allowed to stir for 2 days to provide the acetylated product. The reaction was quenched by addition of saturated $NaHCO_3$, which was stirred overnight. The solution was then evaporated and stirred with more $NaHCO_3$ solution overnight. This was extracted with ether which was washed (carefully) twice with sat. $NaHCO_3$ and once with water, dried over $MgSO_4$ and Norit®, vacuum filtered through celite®, and evaporated. The product is a dark yellow, clear liquid. Gas chromatography (Init. T—80°; Init. time=5 min.; Prog. rate—10°/min; Final T=240° C.) typically indicates a purity of approximately 70%.

Silylation of 5-Fluorocytosine

5-Fluorocytosine (1.05 equivalents based on amount of acetylated lactol obtained in the previous step using GC indication of purity) was silylated by reflux in at least 10 equivalents of hexamethyldisilazane containing a catalytic amount of pure ammonium sulfate (0.05 to 0.10 eq.) for two hours after the solution turned clear. The flask was then sealed tightly and the solvent removed using a vacuum pump with an auxiliary trap. The product, a white solid, was left under vacuum over night until ready for use in the following coupling reaction.

Coupling of Silylated 5-Fluorocytosine with Acetylated Lactol

To silylated 5-fluorocytosine (33.86 gm. 0.124 mol) in dry dichloromethane (350 ml) was added $SnCl_4$ solution (135.6 ml, a 1 molar solution in CH_2Cl_2) under nitrogen atmosphere. The solution was stirred for 15 minutes at room temperature. This solution was cannulated to the solution of the lactol acetate (38 gm, 0.113 mol) in dichloromethane (400 ml) under nitrogen atmosphere over a period of 30 minutes.

The reaction solution was stirred for 2 hours, at which point the completion of reaction was indicated by TLC. The reaction solution was then diluted with dichloromethane (500 ml) and quenched with ammonium hydroxide solution. The ammonium hydroxide solution (100 ml) was added slowly maintaining the temperature of reaction below 30° C., resulting in the formation of a white precipitate.

The mixture was allowed to stir for another 30 minutes, and then passed through silica gel plug column (7 inch diameter 5 inch height). It was eluted sequentially with dichloromethane (2 L), ethyl acetate (2 L) and ethyl acetate:ethanol (9:1) (4 L). The ethyl acetate and ethyl acetate:ethanol eluents contained the desired product. These solutions were combined and evaporated at reduced pressure. The residual sticky solid was then washed with dry ether (200 ml) to give a white solid (25.35 gm; 71%), FTC-5'-butyrate.

FTC-5'-butyrate (8.74 gm; 0.026 mol) was dissolved in 250 ml methanol. Sodium methoxide (2.85 gm; 0.052 gm) was added at room temperature. The reaction was stirred for 1 hour, at which point the completion of reaction was confirmed by TLC. NH_4Cl solution (10 ml) was added to quench the reaction, and then the solvent was removed under reduced pressure. The residue was absorbed on silica gel (5 gm) and passed through a small column using ethyl acetate:ethanol as an eluent (9:1). The product-containing fractions were combined and evaporated to give a sticky solid which was washed with dry ether to give-white solid FTC (6.00 gm, 88%). (^1H NMR: (DMSO-d^6) 8.18 (1H, d, H_6, J=8.4 Hz), 7.81 & 7.57 (2H, broad, NH_2), 6.12 (1H, dd,

$H_{1'}$, J=5.7 & 4.2 Hz), 5.40 (1H, t, OH, J=5.7 Hz), 5.17 (1H, t, $1H_{4'}$, J=3–6 Hz), 3.74 (2H, m, $2H_{5'}$), 3.41 (1H, dd, $1H_{2''}$, J=5.7 & 11.7 Hz), 3.11 (1H, dd, $1H_{2''}$, J=4.2 & 11.7 Hz); ^{13}C NMR: (DMSO-d^6) 157.85 (d, J=13.4 Hz), 153.28, 136.12 (d, J=241 HZ), 126.01 (d, J=32.6 Hz), 86.90, 86.84, 62.48, 37.07; mp 195–196° C.

III. Resolution of Nucleoside Enantiomers

A method is provided herein for the resolution of racemic mixtures of nucleoside enantiomers, including but not limited to the (+) and (−) enantiomers of FTC. The method can also be used to resolve racemic mixtures of carbohydrates or carbohydrate-like moieties, such as derivatives of 1,3-oxathiolane and 1,3-dioxolane. The method involves the use of an enzyme that preferentially catalyzes a reaction of one enantiomer in a racemic mixture. The reacted enantiomer is separated from the unreacted enantiomer on the basis of the new difference in physical structure. Given the disclosure herein, one of skill in the art will be able to choose an enzyme that is selective for the nucleoside enantiomer of choice (or selective for the undesired enantiomer, as a method of eliminating it), by selecting one of the enzymes discussed below or by systematic evaluation of other known enzymes. Given this disclosure, one of skill in the art will also know how to modify the substrate as necessary to attain the desired resolution. Through the use of either chiral NMR shift reagents, polarimetry, or chiral HPLC, the optical enrichment of the recovered ester can be determined.

The following examples further illustrate the use of enzymes to resolve racemic mixtures of enantiomers. Other known methods of resolution of racemic mixtures can be used in combination with the method of resolution disclosed herein. All of these modifications are considered within the scope of the invention.

Resolution Based on Hydrolysis of C5'-Nucleoside Esters

In one embodiment, the method includes reacting the C5'-hydroxyl group of a mixture of nucleoside racemates with an acyl compound to form C5'-esters in which the nucleoside is in the "carbinol" end of the ester. The racemic mixture of nucleoside C5'-esters is then treated with an enzyme that preferentially cleaves, or hydrolyses, one of the enantiomers and not the other, in a given time period.

An advantage of this method is that it can be used to resolve a wide variety of nucleosides, including pyrimidine and purine nucleosides that are optionally substituted in the carbohydrate moiety or base moiety. The method can also be used to resolve nucleoside derivatives that contain additional heteroatoms in the carbohydrate moiety, for example, FTC and BCH-189. The broad applicability of this method resides in part on the fact that although the carbinol portion of the ester plays a role in the ability of an enzyme to differentiate enantiomers, the major recognition site for these enzymes is in the carboxylic acid portion of the ester. Further, one may be able to successfully extrapolate the results of one enzyme/substrate study to another, seemingly-different system, provided that the carboxylic acid portions of the two substrates are the same or substantially similar.

Another advantage of this method is that it is regioselective. Enzymes that hydrolyse esters typically do not catalyze extraneous reactions in other portions of the molecule. For example, the enzyme lipase catalyses the hydrolysis of the ester of 2-hydroxymethyl-5-oxo-1,3-oxathiolane without hydrolysing the internal lactone. This contrasts markedly with "chemical" approaches to ester hydrolysis.

Still another advantage of this method is that the separation of the unhydrolysed enantiomer and the hydrolysed enantiomer from the reaction mixture is quite simple. The unhydrolysed enantiomer is more lipophilic than the hydrolysed enantiomer and can be efficiently recovered by simple extraction with one of a wide variety of nonpolar organic solvents or solvent mixtures, including hexane and hexane/ether. The less lipophilic, more polar hydrolysed enantiomer can then be obtained by extraction with a more polar organic solvent, for example, ethyl acetate, or by lyophilization, followed by extraction with ethanol or methanol. Alcohol should be avoided during the hydrolysis because it can denature enzymes under certain conditions.

Enzymes and Substrates

With the proper matching of enzyme and substrate, conditions can be established for the isolation of either nucleoside enantiomer. The desired enantiomer can be isolated by treatment of the racemic mixture with an enzyme that hydrolyses the desired enantiomer (followed by extraction of the polar hydrolysate with a polar solvent) or by treatment with an enzyme that hydrolyses the undesired enantiomer (followed by removal of the undesired enantiomer with a nonpolar solvent).

Enzymes that catalyze the hydrolysis of esters include esterases, for example pig liver esterase, lipases, including porcine pancreatic lipase and Amano PS-800 lipase, substillisin, and α-chymotrypsin.

FIG. 3 is a flow chart of the specificity of alkaline phosphatase and snake venom phosphodiesterase for the (+) and (−) enantiomers of FTC. As indicated, alkaline phosphatase hydrolyses the triphosphate of both of the enantiomers to FTC, and therefore is not effective as a separation means. Phosphodiesterase I preferentially hydrolyses the (+)-isomer of FTC to its monoester, which can then be exposed to 5'-nucleotidase to provide (+)-FTC.

The most effective acyl group to be used to esterify the C5'-position of the nucleoside can be determined without undue experimentation by evaluation of a number of homologs using the selected enzyme system. For example, when 1,3-oxathiolane nucleosides are esterified with butyric acid, resolutions with both pig liver esterase and Amano PS-800 proceed with high enantioselectivity (94–100% enantiomeric excess) and opposite selectivity. Pig liver esterase preferentially hydrolyses the (+)-enantiomer of FTC, and Amano PS-800® preferentially hydrolyses the (−)-enantiomer of FTC. The percent enantiomeric excess reported in Table 1 is the amount of purified butyrate ester remaining in the enzyme treated mixture (i.e., the butyrate ester of (−)-FTC in the case of PLE and the butyrate ester of (+)-FTC in the case of Amano PS-800®).

Non-limiting examples of acyl groups that can be evaluated for use with a particular nucleoside enantiomeric mixture and particular enzyme include alkyl carboxylic acids and substituted alkyl carboxylic acids, including acetic acid, propionic acid, butyric acid, and pentanoic acid. With certain enzymes, it may be preferred to use an acyl compound that is significantly electron-withdrawing to facilitate hydrolysis by weakening the ester bond. Examples of electron-withdrawing acyl groups include α-haloesters such as 2-chloropropionic acid, 2-chlorobutyric acid, and 2-chloropentanoic acid. α-Haloesters are excellent substrates for lipases.

Resolution Conditions

The enzymatic hydrolyses are typically carried out with a catalytic amount of the enzyme in an aqueous buffer that has a pH that is close to the optimum pH for the enzyme in question. As the reaction proceeds, the pH drops as a result of liberated carboxylic acid. Aqueous base should be added to maintain the pH near the optimum value for the enzyme. The progress of the reaction can be easily determined by monitoring the change in pH and the amount of base needed

to maintain pH. The hydrophobic ester (the unhydrolysed enantiomer) and the more polar alcohol (the hydrolysed enantiomer) can be sequentially and selectively extracted from the solution by the judicious choice of organic solvents. Alternatively, the material to be resolved can be passed through a column that contains the enzyme immobilized on a solid support.

Enzymatic hydrolyses performed under heterogeneous conditions can suffer from poor reproducibility. Therefore, it is preferred that the hydrolysis be performed under homogeneous conditions. Alcohol solvents are not preferred because they can denature the enzymes. Homogeneity can be achieved through the use of non-ionic surfactants, such as Triton X-100. However, addition of these surfactants not only assists in dissolving the starting material, they also enhance the aqueous solubility of the product. Therefore, although the enzymatic reaction can proceed more effectively with the addition of a non-ionic surfactant than under heterogeneous conditions, the isolation of both the recovered starting material and the product can be made more difficult. The product can be isolated with appropriate chromatographic and chemical (e.g., selective salt formation) techniques. Diacylated nucleosides can be used but are often quite lipophilic and hard to dissolve in the medium used.

EXAMPLE 2

Enantioselective Lipase-Catalyzed Hydrolysis of FTC Esters.

A number of 5'-O-acyl derivatives of FTC were prepared by selective O-acylation of the N-hydrochloride salt (see Table 1 and FIG. 4) of (±)-FTC. The efficiency of the hydrolysis of the derivatives by lipases was investigated. As shown in Table 1, pig liver esterase (PLE) exhibits a high level of selectivity for the hydrolysis of the ester of the (+)-enantiomer of FTC, leaving predominately the butyrate of (−)-FTC in the HPLC-analyzed mixture. In contrast, PS-800 hydrolyses the ester of the (−)-enantiomer of FTC preferentially, leaving predominately the butyrate of the (+)-FTC in the HPLC-analyzed mixture. The rate of the hydrolysis was also found to be dependent on the nature of the acyl group; the acetyl derivative was significantly slower than the butyryl derivative. It has now been discovered that the rate of the hydrolysis of the propionic acid ester of FTC is even faster than that observed for the butyrate derivative. Percent recovery and percent of enantiomeric excess were both determined using HPLC. Although the enantioselectivity is excellent when employing PLE (typically 97% e.e. or higher), additional enrichment can be accomplished by sequential enzymatic hydrolysis reactions in which the enantiomerically-enriched butyrate from a PLE-catalyzed hydrolysis is subjected to enzymatic hydrolysis by PS-800.

TABLE 1

Comparison of Effect of Ester on Enzyme Hydrolysis

Substrate	% Recovery	% E.E. (s.m.)
FTC Esters with PLE: (butyrate)		(−)-FTC
acetate	32.68	N.D.
propionate	39.87	N.D.
butyrate	48.00	98
butyrate	45.71	98.6
FTC Esters with PS800:		(+)-FTC
acetate	73.17	N.D.

TABLE 1-continued

Comparison of Effect of Ester on Enzyme Hydrolysis

Substrate	% Recovery	% E.E. (s.m.)
propionate	52.67	N.D.
butyrate	58.34	N.D.
valerate	41.50	94

EXAMPLE 3

Procedure for the Preparation of (+)- and (−)-FTC via Enantioselective, Lipase-Catalyzed Hydrolysis of FTC Butyrate.

The 5'-O-butyrate of (±)-FTC (0.47 mmol, 149 mg) was dissolved in 16 mL of a solution of 4:1 pH 8 buffer:CH_3CN. The clear solution was stirred and treated with 26 mg of pig liver esterase (PLE-A). The progress of the reaction was monitored by HPLC (FIG. 4). After 20 hours (52% conversion), the reaction mixture was extracted with 2×80 mL of $CHCl_3$ and 80 mL of ethyl acetate. The organic layer extracts were combined, dried over anhydrous $MgSO_4$, filtered, and concentrated by rotary evaporation. The resulting residue was eluted on 2×1000 m pTLC plates using ethyl acetate as eluant (double elution) to give, after isolation, 53 mg (36% based on starting material) of FTC butyrate which was determined to have 98% enantiomeric excess (e.e.) by HPLC analysis. The enantiomerically-enriched butyrate was then treated with 1.6 mL of methanol followed by 0.38 mmol (20 mg) of sodium methoxide. The resulting mixture was stirred at room temperature, and the progress of the reaction was monitored by HPLC. The reaction was completed within 30 minutes. The solvent was removed by rotary evaporation to give a crude white solid (76 mg) that was eluted on a 1000 m pTLC using 5:1 ethyl acetate:ethanol. (−)-FTC was isolated as a white solid (33 mg; 82% yield). HPLC analysis of the FTC as its 5'-O-acetate derivative showed 97% e.e.; $[\alpha]({}^{20}{}_{,D})$ −120.5° (c=0.88; abs. ethanol).

Emulsions in the work-up step can be avoided by adding $HCCl_3$ to the reaction mixture on completion (which also serves to denature the enzyme), stripping the solvents under vacuum, and then extracting with $HCCl_3$.

Similarly, 1.2 mmol (375 mg) of the 5'-O-butyrate of (±)-FTC was dissolved in 40 mL of 4:1 pH 8 buffer-CH_3CN. The clear solution was stirred and treated with 58 mg of pig liver esterase (PLE-A). The progress of the reaction was monitored by HPLC. After 90 minutes (38% conversion), the reaction mixture was added to 150 mL of $CHCl_3$. The layers were separated and the aqueous layer lyophilized to remove solvent. The white residue from the lyophilization was extracted with 3×10 mL of absolute ethanol. The extracts were filtered, combined, and concentrated in vacuo to yield 179 mg of crude oil. The crude material was eluted on a 45×30 mm column of silica gel using 3×75 mL of ethyl acetate followed by 5:1 ethyl acetate-ethanol. (+)-FTC was isolated as a white solid (109 mg; 37% based on starting butyrate). HPLC analysis of the (+)-FTC as its 5'-O-acetate derivative showed 97.4% e.e.; $[\alpha]O({}^{20}{}_{,D})$ +113.4° (c=2.53; absolute ethanol).

A similar reaction was performed using 0.12 mmol (37 mg) of the 5'-O-butyrate of FTC and 7 mg of PS-800 in 4.0 mL of 4:1 pH 8 buffer:CH_3CN. The reaction was considerably slower than that with PLE-A and required 74 hours for 59% conversion. The recovered butyrate (11.4 mg; 31% of the initial amount) was found to exhibit 94% e.e. by HPLC.

Resolution of Nucleoside Enantiomers with Cytidine-Deoxycytidine Deaminase

In an alternative embodiment, cytidine-deoxycytidine deaminase is used to resolve racemic mixtures of 2-hydroxymethyl-5-(cytosin-1-yl)-1,3-oxathiolane and its derivatives, including 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane. The enzyme catalyses the deamination of the cytosine moiety to a uracil. It has been discovered that one of the enantiomers of 1,3-oxathiolane nucleosides is a preferred substrate for cytidine-deoxycytidine deaminase. The enantiomer that is not converted to a uracil derivative (and therefore is still basic) is extracted from the solution with an acidic solution. Care should be taken to avoid strong acidic solutions (pH below 3.0), that may cleave the oxathiolane ring.

Cytidine-deoxycytidine deaminase can be isolated from rat liver or human liver, or expressed from recombinant sequences in a procaryotic system such as in *E. coli.*

The method of resolution of cytidine nucleoside enantiomers using cytidine-deoxycytidine deaminase can be used as the sole method of resolution or can be used in combination with other methods of resolution, including resolution by enzymatic hydrolysis of 5'-O-nucleoside esters as described above.

Combination of Enzymatic Resolution with Classical Resolution Methods

The process described above for resolving racemic mixtures of nucleoside enantiomers can be combined with other classical methods of enantiomeric resolution to increase the optical purity of the final product.

Classical methods of resolution include a variety of physical and chemical techniques. Often the simplest and most efficient technique is recrystallization, based on the principle that racemates are often more soluble than the corresponding individual enantiomers. Recrystallization can be performed at any stage, including on the acylated compounds or the final enantiomeric product. If successful, this simple approach represents a method of choice.

When recrystallization fails to provide material of acceptable optical purity, other methods can be evaluated. If the nucleoside is basic (for example, a cytidine) one can use chiral acids that form diastereomeric mixtures that may possess significantly different solubility properties. Nonlimiting examples of chiral acids include malic acid, mandelic acid, dibenzoyl tartaric acid, 3-bromocamphor-8-sulfonic acid, 10-camphorsulfonic acid, and di-p-toluoyltartaric acid. Similarly, acylation of the free hydroxyl group with a chiral acid derivative also results in the formation of diastereomeric mixtures whose physical properties may differ sufficiently to permit separation.

Small amounts of enantiomerically enriched nucleosides can be obtained or purified by passing the racemic mixture through an HPLC column that has been designed for chiral separations, including cyclodextrin bonded columns marketed by Rainin Corporation.

EXAMPLE 4

Separation of Racemic Mixtures of Nucleosides by HPLC.

The resolutions of the C4'-enantiomers of (±)-FTC were performed using a chiral cyclodextrin bonded (cyclobond AC-I) column obtained from Rainin Corporation (Woburn, Mass.). The conditions were as follows: Isocratic 0.5% methanol in water; flow rate 1 ml/min., UV detection at 262 nm. HPLC grade methanol was obtained from J. T. Baker (Phillipsburg, N.J.). The racemic mixtures were injected and fractions were collected. Fractions containing each of the enantiomers were pooled, frozen, and then lyophilized. The compounds were characterized by UV spectroscopy and by their retention times on HPLC. In general, the (-)-enantiomers have lower retention times than the (+)-enantiomers (see *J. Liquid Chromatography* 7:353–376, 1984). The concentrations of the compounds were determined by UV spectroscopy, using a stock solution of known concentration (15 μM) prepared in water for biological evaluation. The retention times for the separated enantiomers are provided in Table 2.

TABLE 2

Retention Times of Enantiomers of FTC

Compound	R_t (min)
(-)-FTC	8.3
(+)-FTC	8.7

EXAMPLE 5

Alternative Methods for Separating FTC Enantiomers using a Chiral Column

Using a Cyclobond I-Ac column (5 μm, 25 cm×4.6 mm, Rainin Corporation, Woburn, Mass., catalog no. AST-41049), with a flow rate of 0.6 ml/min of 0.5% isocratic methanol (Fisher Scientific, Inc. HPLC grade, cat no. A-452-4 in water), and UV detection at 262 nm, the FTC enantiomers exhibited retention times of 12.68 minutes ((-)-FTC) and 13.20 minutes ((+)-FTC).

Using a Chiralpak AS column (10 μm, 25 cm×4.6 mm, J. T. Baker Inc., Phillisburg, N.J., catalog no. 7406-00, serial no. 09-29-10320) with a flow rate of 0.8 ml/min of isopropyl alcohol (HPLC grade, Fisher Scientific, Inc., cat no. A-451-4) and UV detection of 262 nm, the FTC enantiomers exhibited retention times of 5.9 minutes ((-)-FTC), and 9.8 minutes ((+)-FTC)

IV. Ability of 2-Hydroxymethyl-5-(5-Fluorocytosin-1-yl)-1, 3-Oxathiolane ("FTC") to Inhibit the Replication of HIV

It is often desirable to screen a number of racemic mixtures of nucleosides as a preliminary step to determine which warrant further resolution into enantiomerically enriched components and further evaluation of antiviral activity. The ability of nucleosides to inhibit HIV can be measured by various experimental techniques. The technique used herein, and described in detail below, measures the inhibition of viral replication in phytohemagglutinin (PHA) stimulated human peripheral blood mononuclear (PBM) cells infected with HIV-1 (strain LAV). The amount of virus produced is determined by measuring the virus-coded reverse transcriptase enzyme. The amount of enzyme produced is proportional to the amount of virus produced. Table 3 provides the EC_{50} values (concentration of nucleoside that inhibits the replication of the virus by 50% in PBM cells, estimated 10% error factor) and IC_{50} values (concentration of nucleoside that inhibits 50% of the growth of mitogen-stimulated uninfected human PBM cells) of a number of (+)-1,3-oxathiolane and nucleosides.

EXAMPLE 6

Anti-HIV Activity of (±)-1,3-Oxathiolane Nucleosides.

A. Three-day-old phytohemagglutinin-stimulated PBM cells (10^6 cells/ml) from hepatitis B and HIV-1 seronegative healthy donors were infected with HIV-1 (strain LAV) at a concentration of about 100 times the 50% tissue culture infectious dose (TICD 50) per ml and cultured in the presence and absence of various concentrations of antiviral compounds.

B. Approximately one hour after infection, the medium, with the compound to be tested (2 times the final concentration in medium) or without compound, was added to the flasks (5 ml; final volume 10 ml). AZT was used as a positive control.

C. The cells were exposed to the virus (about 2×10^5 dpm/ml, as determined by reverse transcriptase assay) and then placed in a CO_2 incubator. HIV-1 (strain LAV) was obtained from the Center for Disease Control, Atlanta, Ga. The methods used for culturing the PBM cells, harvesting the virus and determining the reverse transcriptase activity were those described by McDougal et al. (*J. Immun. Meth.* 76, 171–183, 1985) and Spira et al. (*J. Clin. Meth.* 25, 97–99, 1987), except that fungizone was not included in the medium (see Schinazi, et al., *Antimicrob. Agents Chemother.* 32, 1784–1787 (1988); Id., 34:1061–1067 (1990)).

D. On day 6, the cells and supernatant were transferred to a 15 ml tube and centrifuged at about 900 g for 10 minutes. Five ml of supernatant were removed and the virus was concentrated by centrifugation at 40,000 rpm for 30 minutes (Beckman 70.1 Ti rotor). The solubilized virus pellet was processed for determination of the levels of reverse transcriptase. Results are expressed in dpm/ml of sampled supernatant. Virus from smaller volumes of supernatant (1 ml) can also be concentrated by centrifugation prior to solubilization and determination of reverse transcriptase levels.

The median effective (EC_{50}) concentration was determined by the median effect method (*Antimicrob. Agents Chemother.* 30, 491–498 (1986). Briefly, the percent inhibition of virus, as determined from measurements of reverse transcriptase, is plotted versus the micromolar concentration of compound. The EC_{50} is the concentration of compound at which there is a 50% inhibition of viral growth.

E. Mitogen stimulated uninfected human PBM cells (3.8×10^5 cells/ml) were cultured in the presence and absence of drug under similar conditions as those used for the antiviral assay described above. The cells were counted after 6 days using a hemacytometer and the trypan blue exclusion method, as described by Schinazi et al., *Antimicrobial Agents and Chemotherapy*, 22(3), 499 (1982). The IC_{50} is the concentration of compound which inhibits 50% of normal cell growth.

TABLE 3

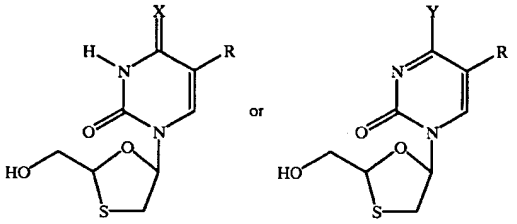
EC_{50} and IC_{50} of Various Analogues of 1,3-Oxathiolane Nucleosides in Human PBM Cells

			Antiviral Cytotoxicity	
Code	X or Y	R	EC_{50}, μM	IC_{50}, μM
DLS-009	X = O	H	>100	>100
DLS-010	X = O	Me	64.4	>100
DLS-027	X = O	F	>100	>100
DLS-028	X = O	Cl	60.8	>100
DLS-044	X = O	Br	>100	>100
DLS-029	X = O	I	>100	>100
DLS-020	Y = NH_2	H	0.02	>100
DLS-011	Y = NH_2	Me	>10	>100
DLS-022	Y = NH_2	F	0.01	>100
DLS-023	Y = NH_2	Cl	38.7	>100
DLS-021	Y = NH_2	Br	77.4	>100
DLS-026	Y = NH_2	I	0.72	>100
DLS-058(-)	Y = NH_2	F	0.008	>100

TABLE 3-continued

EC_{50} and IC_{50} of Various Analogues of 1,3-Oxathiolane Nucleosides in Human PBM Cells

			Antiviral Cytotoxicity	
Code	X or Y	R	EC_{50}, μM	IC_{50}, μM
DLS-059(+)	Y = NH_2	F	0.84	>100
DLS-053	Y = NH_2	CF_3	60.7	>100



As indicated in Table 3, in general, the substituted cytosine 1,3-oxathiolane nucleosides are more active than the corresponding uracil nucleosides. The error in EC_{50} and IC_{50} measurements are estimated at ±10%.

One of the compounds, (±)-FTC, (referred to as "DLS-022", compound 8) not only exhibits exceptional activity (approximately 10 nM in PBM cells), but also quite low toxicity (>100 μM in PBM, Vero and CEM cells). Further, the (−)-enantiomer of FTC (DLS-058), exhibits significantly greater activity than the racemic mixture.

The IC_{50} of (+)-FTC was over 100 μM, indicating that the compound was not toxic in uninfected PBM cells evaluated up to 100 μM.

EXAMPLE 7

Antiviral Activity of the Enantiomers of FTC Resolved by HPLC.

The enantiomers of FTC were isolated by the method of Example 4, and the antiviral activity evaluated by the method of Example 6. The results are provided in Table 4, and illustrated in FIG. 5.

TABLE 4

Antiviral Activity of the (+) and (−) Enantiomers of FTC

Treatment	Concn., μM	DPM/ml	% Inhibition (Corrected)	EC_{50};μM
FTC (±)	0.0001	73,755	26.6	0.018
	0.005	83,005	16.3	
	0.01	60,465	41.3	
	0.05	34,120	70.4	
	0.1	14,160	92.4	
	0.5	18,095	88.1	
	1	7,555	99.7	
	5	7,940	99.3	
	10	5,810	101.7	
FTC (−)	0.001	76,275	23.8	0.02
	0.005	58,590	43.3	
	0.01	75,350	24.8	
	0.05	28,890	76.2	
	0.1	13,175	93.5	
	0.5	9,485	97.6	
FTC (+)	0.001	94,340	3.8	0.28
	0.005	107,430	−10.6	
	0.01	99,465	−1.8	
	0.05	87,120	11.8	

TABLE 4-continued

Antiviral Activity of the (+) and (−) Enantiomers of FTC

Treatment Concn., μM	DPM/ml	% Inhibition (Corrected)	EC_{50};μM
0.1	86,340	12.7	
0.5	33,225	71.4	

As indicated in Table 4, in this experiment the (−)-enantiomer of FTC appears to be approximately one order of magnitude more potent than the (+)-FTC enantiomer, and has approximately the same anti-HIV activity as the racemic mixture. Neither the enantiomers nor the racemic mixture is toxic up to 100 μM as measured by the Trypan Blue exclusion method in human PBM cells.

EXAMPLE 8

Antiviral Activity of FTC Enantiomers Resolved by Method of Example 3.

The enantiomers of (±)-FTC were also resolved by the method of Example 3, and the antiviral activity evaluated by the method of Example 6. The results are illustrated in FIG. 6. As indicated in FIG. 6, the EC_{50} of the racemic mixture of FTC was 0.017 μM, the EC_{50} of (−)-FTC at 0.0077 μM, and the EC_{50} of (+)-FTC at 0.084 μM.

EXAMPLE 9

Uptake of (±)-FTC into Human PBM Cells

Studies were undertaken using radiolabeled FTC to follow the intracellular profiles of the parent drug and metabolites detected within the cell. All studies were conducted in duplicate. Human peripheral blood mononuclear cells (PBM cells) were suspended in RPMI 1640 medium containing 10% fetal calf serum and antibiotics (2×10^6 cells/ml), 10 ml per timepoint) and incubated with addition of 10 μM FTC (specific activity about 700 dpm/pmol). Cells were exposed to the drug for 2, 6, 12, and 24 hours. At these timepoints, the medium was removed and the cells were washed two times with cold Hank's balanced salt solution. Extraction was performed with addition of 0.2 ml of 60% cold methanol/water and stored overnight at −70° C. The following morning, the suspensions were centrifuged and extractions were repeated two times for 0.5 hours at −70° C. The total supernatants (0.6 ml) were lyophilized to dryness. The residues were resuspended in 250 μl of water and aliquots of between 50 and 100 μl were analyzed by HPLC. Quantitation of intracellular parent drug and metabolic derivatives were conducted by HPLC. Because of the potential acid lability of some compounds, a buffer system close to physiological pH was used for the separation of the metabolites.

FIG. 7 is a graph of the presence (uptake) of tritiated (+)-FTC in human PBM cells (average of two determinations) in time (hours) versus pmol/10^6 cells. The uptake studies indicate that radiolabeled FTC is readily taken up in human lymphocytes, that produce very large amounts of the 5'-triphosphate derivative of FTC.

EXAMPLE 10

Antiretroviral Activity of FTC in Various Cell Lines

The antiretroviral activity of FTC was measured in a number of cell lines using procedures similar, but not identical, to that set out in Example 6. Cell lines were obtained from either human donors, AIDS Research and Reference Reagent Program, NIH, Rockville, Md., ATCC, or the Red Cross. The CEM thymidine kinase deficient cells were prepared by sequential passage of CEM cells in the presence of 5-bromo-2'-deoxyuridine. The results are provided in Table 5.

TABLE 5

Antiretroviral Activity of FTC In Different Cell Systems

Cell system (Virus strain)	EC_{50} (μM) (±)-FTC
HIV-1	
PBMC (LAV-1)	0.027
MT2 ($HTLV_{IIIB}$)	0.89
CEM (LAV-1)	0.08
CEM-$TK^{(-)}$ (LAV-1)	0.026
CEM ($HTLV_{IIIB}$)NIH	0.09
HIV-2	
PBMC (ROD2)	0.0038 (±)-FTC 0.0007 (−)-FTC 0.026 (+)-FTC
SIV	
AA-2 (SIV251)	4.6
C-8166 (SIV251)	<8.0
FIV	
CrFK (61E)	≦1

EXAMPLE 11

Egress of (+)-FTC from Human PBM Cells.

Studies were performed using radiolabeled FTC to follow the intracellular profiles of the parent drug and metabolites detected within the cell after incubation in media with drug for 24 hours, and then removal of drug. This study measures the time needed for intracellular levels of triphosphates to decline. Studies were conducted in duplicate. Uninfected cells (2×10^6 ml) were suspended in the appropriate medium supplemented with serum (10 ml per timepoint) and incubated at 37° C. in a 5% CO_2 incubator. The radiolabeled FTC concentration was 10 μM. After pulsing the cells with the labeled compound for 24 hours, the cells were thoroughly washed and then replenished with fresh medium without the antiviral drugs (0 hr). At 0, 2, 4, 6, 12, 24, and 48 hours (second incubation time), the cells were removed, and immediately extracted with 60% cold methanol/water. The extract was obtained by centrifugation and removal of the cell pellet. The extracts were lyophilized and then stored at −70° C. Prior to analysis, the material was resuspended in 250 microliters of HPLC buffer and immediately analyzed. Quantitation of intracellular parent drug and metabolic derivatives was conducted by HPLC, using either a Micromeritics or Hewlett-Packard model 1090 PHLC system with an anion exchange Partisil 10 SAX column (Whatman, Inc.), at a flow rate of 1 ml/min, 1 kpsi pressure, with UV detection at 262 nm. The mobile phase consisted of deionized water (A), 2 mM NaH_2PO_4/16 mM NaOAc (pH=6.6) (B), 15 mM NaH_2PO_4/120.2 mM NaOAc (pH=6.6) (C), and 100 mM NaH_2PO_4/800 mM NaOAc (pH=6.6) (D).

Separation method: isocratic for 5 minutes with A, followed by a 15 minute linear gradient to 100% B, followed by a 20 minute linear gradient to 100% C, followed by 10 minute linear gradient to 100% D, followed by 30 minutes isocratic with 100% D.

Retention times (minutes) in Human Cells:

Compound	Unchanged	Mono-phosphate	Diphosphate	Triphosphate
(±)-FTC	5.0	39.0	55.0	68.0

FIG. 8 is a graph of the egress of radiolabeled (+)-FTC from human PBM cells, measured in hours after drug removal versus concentration (pmol/10^6 cells). As indicated in the Figure, FTC-triphosphate has an intracellular half-life of approximately 12 hours and can be easily detected intracellularly at concentrations of 1–5 μM 48 hours after the removal of the extracellular drug, which is well above the EC_{50} for the compound. Further, the affinity (K^I) for (±)-FTC triphosphate using HIV RT is 0.2 μM, which is below the 48 hour concentration level.

EXAMPLE 12

Anti-HIV Activity of Pharmaceutically Acceptable Derivatives of (±)-FTC

a. A number of pharmaceutically acceptable derivatives of (±)-FTC prepared by derivatizing the 5' and N^4 positions were evaluated for anti-HIV activity in PBM cells using a procedure similar to that described in Example 6. The results are as follows. The 5'-O-butyrate ester of (±)-FTC exhibited an EC_{50} of 0.0017. The N^4-acetyl derivative of (±)-FTC exhibited an EC_{50} of 0.0028. The 5'-O-butyrate, N^4-ester of (±)-FTC exhibited an EC_{50}=0.0058.

b. The anti-HIV activity of the 5'-O-butyrate ester of (±)-FTC in the MT4 system (EC_{50}) was 0.04 μM. In the same assay, the unacylated (±)-FTC exhibited an IC50 of 0.52 μM. The IC50 for AZT in this system was 0.09 μM.

V. Ability of FTC to Inhibit the Replication of HBV

EXAMPLE 13

Evaluation of Activity of (+) and (−)-Enantiomers of FTC in 2.2.15 Cell Cultures

The ability of the enantiomers of FTC to inhibit the growth of virus in 2.2.15 cell cultures (HepG2 cells transformed with hepatitis virion) is described in detail below.

A summary and description of the assay for antiviral effects in this culture system and the analysis of HBV DNA has been described (Korba and Milman, 1991, *Antiviral Res.*, 15:217). The antiviral evaluations were performed on two separate passages of cells. All wells, in all plates, were seeded at the same density and at the same time.

ASSAY PARAMETERS

Due to the inherent variations in the levels of both intracellular and extracellular HBV DNA, only depressions greater than 3.5-fold (for HBV virion DNA) or 3.0-fold (for HBV DNA replication intermediates) from the average levels for these HBV DNA forms in untreated cells are considered to be statistically significant [$P<0.05$]. The levels of integrated HBV DNA in each cellular DNA preparation (which remain constant on a per cell basis in these experiments) were used to calculate the levels of intracellular HBV DNA forms, thereby ensuring that equal amounts of cellular DNA were compared between separate samples.

Typical values for extracellular HBV virion DNA in untreated cells ranged from 50 to 150 pg/ml culture medium (average of approximately 76 pg/ml). Intracellular HBV DNA replication intermediates in untreated cells ranged from 50 to 100 pg/μg cell DNA (average approximately 74 pg/μg cell DNA). In general, depressions in the levels of intracellular HBV DNA due to treatment with antiviral compounds are less pronounced, and occur more slowly, than depressions in the levels of HBV virion DNA (Korba and Milman, 1991, *Antiviral Res.*, 15:217).

The manner in which the hybridization analyses were performed for these experiments resulted in an equivalence of approximately 1.0 pg of intracellular HBV DNA to 2–3 genomic copies per cell and 1.0 pg/ml of extracellular HBV DNA to 3×10^5 viral particles/ml.

TOXICITY ANALYSIS

Toxicity analyses were performed to assess whether any observed antiviral effects were due to a general effect on cell viability. The method used herein was the measurement of the uptake of neutral red dye, a standard and widely used assay for cell viability in a variety of virus-host systems, including HSV and HIV. Toxicity analyses were performed in 96-well flat bottomed tissue culture plates. Cells for the toxicity analyses were cultured and treated with test compounds with the same schedule as described for the antiviral evaluations below. Each compound was tested at 4 concentrations, each in triplicate cultures (wells "A", "B", and "C"). Uptake of neutral red dye was used to determine the relative level of toxicity. The absorbance of internalized dye at 510 nm (A_{sin}) was used for the quantitative analysis. Values are presented as a percentage of the average A_{sin} values in 9 separate cultures of untreated cells maintained on the same 96-well plate as the test compounds. Dye uptake in the 9 control cultures on plate 5 ranged from 91.6% to 110.4%, and on plate 6 from 96.6% to 109%. The results are provided in Table 6.

TABLE 6

Toxicity Analysis of Test Compounds in 2.2.15 Cells

		CONC.	DYE UPTAKE (% OF CONTROL)		
			WELL		
PLATE	COMPOUND	(μM)	WELL A	B	WELL C
5	DMSO	10.0*	0.7	1.6	0.9
		3.3	55.9	68.7	61.7
		1.0	91.2	96.4	106.8
		0.3	98.7	102.9	93.5
6	(−)-FTC	300	53.0	51.1	51.5
		100	64.1	66.6	77.6
		30	98.7	94.3	96.4
		10	94.3	94.9	92.2
6	(+)-FTC	300	43.4	56.7	58.5
		100	77.7	66.3	72.1
		30	81.1	88.3	88.1
		10	90.9	99.4	90.5

* For DMSO, concentrations are presented as percent of original stock solution.

TOXICITY EVALUATION

As indicated in Table 6, no significant toxicity (greater than 50% depression of the dye uptake levels observed in untreated cells) was observed for the test compounds at the concentrations used for the antiviral evaluations. Both test compounds, (−)-FTC and (+)-FTC, appeared to be toxic at the highest concentration used for the toxicity tests (330 μM).

ANTIVIRAL EVALUATIONS

CONTROLS

Within normal variations, levels of HBV virion DNA and intracellular HBV replication intermediates [HBV RI] remained constant in the untreated cells over the challenge period. DMSO, at a concentration of 1%, did not affect the levels of HBV replication in 2.2.15 cell cultures.

TEST COMPOUNDS

As indicated in Table 7, both (−)-FTC and (+)-FTC significantly inhibited the replication of HBV at the tested levels. As indicated in Table 8, (−)-FTC still significantly

inhibits the synthesis of HBV virion DNA and intracellular HBV DNA at concentrations of 4, 1, and 0.25 μm.

TABLE 7

Effect of Test Compounds on HBV Production In 2.2.15 Cell Cultures

		HBV Virion DNA* (pg/ml Culture Medium)			Intracellular HBV DNA (pg/μg Cell DNA)	
WELL	TREATMENT	DAY 0	DAY 4	DAY 9	MONO.	RI
7A	Untreated Cells	59	75	94	2.7	93
7B	Untreated Cells	47	64	88	2.5	93
8A	Untreated Cells	65	100	71	2.2	97
8B	Untreated Cells	77	65	110	2.4	62
7K	DMSO @ 1.00%	100	50	48	1.9	95
7L	DMSO @ 1.00%	48	96	54	2.8	98
8K	DMSO @ 1.00%	93	63	68	2.2	86
8L	DMSO @ 1.00%	66	57	59	1.6	97
9U	(-)-FTC @10 μM	120	36	1	1.1	14
9V	(-)-FTC @10 μM	89	48	1	1.5	19
10U	(-)-FTC @10 μM	58	41	0.1	1.9	13
10V	(-)-FTC @10 μM	110	32	0.1	1.2	16
9W	(+)-FTC @10 μM	88	42	0.1	0.8	14
9X	(+)-FTC @10 μM	58	57	0.2	0.4	19
10w	(+)-FTC @10 μM	69	55	0.1	0.7	17
10X	(+)-FTC @10 μM	45	39	0.1	0.4	15

*Sensitivity cutoff for HBV virion DNA was 0.1 pg/ml.
@Intracellular HBV DNA was analyzed 24 hours following the 9th day of treatment. The levels of integrated HBV DNA in each cell DNA preparation were used to calculate the levels of episomal 3.2 Kb HBV genomes (MONO.) and HBV DNA replication intermediates (RI).

TABLE 8

Effect of Test Compounds on HBV Production in 2.2.15 Cell Cultures

		HBV VIRION DNA* (pg/ml CULTURE MEDIUM)			INTRA-CELLULAR HBV DNA* (pg/μg CELL DNA)	
WELL	TREATMENT	DAY 0	DAY 4	DAY 9	MONO.	RI
31A	untreated cells	64	54	65	2.8	65
31B	"	51	54	77	2.0	53
32A	"	100	76	56	3.5	81
32B	"	53	97	83	3.1	68
35A	(-)-FTC @ 4 μM	74	27	>0.1	1.4	1
35B	"	87	28	>0.1	0.5	1
36A	"	120	20	1	0.9	1
36B	"	59	16	0.2	0.2	2
35C	(-)-FTC @ 1 μM	70	13	>0.1	1.7	2
35D	"	62	15	>0.1	1.2	3
36C	"	60	22	1	1.4	2
36D	"	89	28	0.3	1.5	4
35E	(-)-FTC @ 0.25 μM	84	15	>0.1	1.5	4
35F	"	89	16	4	2.2	4
36E	"	66	13	1	1.8	8
36F	"	49	19	0.1	0.3	9

* Sensitivity cutoff for HBV virion DNA was 0.1 pg/ml.
+Analysis of intracellular HBV DNA was 24 hours following the 9th day of treatment. The levels of integrated HBV DNA in each cell DNA preparation were used to calculate the levels of episomal 3.2 kb HBV genomes (MONO.) and HBV DNA replication intermediates (RI).

EXAMPLE 14

Uptake of (±)-FTC into Human Liver Cells; HVB Activity of FTC.

The procedure of Example 9 was repeated with human liver cells (HepG2 cells, available from the ATCC) to determine the uptake and metabolism of FTC in these cells. As shown in FIG. **9**, (±)-FTC is taken up by HepG2 cells in large amounts. These human liver cells metabolize a large percentage of the (±)-FTC to (±)-FTC triphosphate.

This data, in conjunction with other data provided herein, indicate that (±)-FTC, as well as its (-) and (+) enantiomers, are phosphorylated in liver cells. These cells can be transformed with hepatitis B virus.

EXAMPLE 15

Egress of FTC in Human HepG2 cells

FIG. **10** illustrates the egress of [^{3}H]-(±)-FTC and its phosphorylated derivatives in human HepG2 in pmol/10^6 cells over time cells after pulsing cells with 10 μM [^{3}H]-(±)-FTC (700 DPM/pmole) for 24 hours, and evaluating the concentration of compound 24 hours after removal.

FIG. **11** illustrates the decrease in the combined concentration of [^{3}H]-(±)-FTC and its phosphorylated derivatives from human HepG2 cells after incubation with 10 μM [^{3}H]-(±)-FTC (700 DPM/pmole) for 24 hours, in pmol/10^6 cells over time.

As illustrated, even at 48 hours, over 1 μM of active compound (which is significantly higher than the EC_{50} for the compound) is still present in the cells.

V. Toxicity in Granulocyte-Macrophage Precursor Cells

EXAMPLE 16

Effect of FTC on Colony Formation of Granulocyte-Macrophage Precursor Cells

FIG. **12** is a graph of the effect of the (-) and (+) enantiomers of FTC on colony formation of granulocytes-macrophage precursor cells, as measured in percent survival versus concentration in μM ((-)-FTC, open circle; (+)-FTC, darkened circle; AZT, darkened square. As indicated, the (-)-enantiomer of FTC appears to be less toxic, i.e., have a higher IC_{50}, than either the (+)-enantiomer or AZT in this cell line.

VI. Pharmacokinetics of FTC

EXAMPLE 17

Metabolism of FTC on Administration to Rats

(±)-FTC was administered intravenously at dosages of 10, 50 and 100 mg/kg to rats, and the area under the plasma drug concentration versus time (AUC), total clearance (CL_T), steady-state volume of distribution (V_{SS}), mean residence time (MRT) and half-life ($t_{1/2}$), evaluated. The results are provided in Table 9.

TABLE 9

Pharmacokinetic Parameters of FTC After Intravenous Administration of 10, 50, 100 mg/kg to Rats*.

Dose mg/kg	AUC mg h/L	CL_T L/h/kg	V_{SS} L/kg	MRT h	$t_{1/2}$ h
10	9.65	0.988	0.758	0.768	0.757
50	57.11	0.874	0.699	0.800	0.815
100	120.72	0.830	0.663	0.798	0.969

*AUC = area under the plasma drug concentration versus time curve; CL = total clearance; V_{SS} = steady-state volume of distribution; MRT = mean residence time; and $t_{1/2}$ = half-life.

EXAMPLE 18

Pharmacokinetic Parameters for FTC after Intravenous and Oral Administration of FTC

Model-independent pharmacokinetic parameters were derived for (+)-FTC by administration (intravenous (I.V.) and oral (P.O.)) of 33.3 mg/kg to Rhesus Monkeys. The

results are provided in Table 10. Importantly, the mean bioavailability of the compound in monkeys was 73% (±6).

TABLE 10

Model-Independent Pharmacokinetic Parameters Derived for FTC After Intravenous (I.V.) or Oral (P.O.) Administration of 33.3 mg/kg to Rhesus Monkeys*

Monkey	AUC mg h/L	CL_T L/h/kg	V_{SS} L/kg	MRT h	$t_{1/2}$ h	K_a h^{-1}	F %
I.V.							
RUh	19.14	1.74	2.71	1.56	1.28		
RMi	26.31	1.26	1.97	1.56	1.22		
RJd	22.51	1.48	2.00	1.36	1.47		
Mean	22.65	1.49	2.23	1.49	1.32		
±S.D.	3.59	0.24	0.42	0.12	0.13		
P.O.							
RUh	13.21			2.07	1.58	0.48	71
RMi	21.11			2.32	1.08	0.43	80
RJd	15.29			3.23	1.47	0.31	68
Mean	16.54			2.54	1.38	0.41	73.00 (±6)
±S.D.	4.09			0.61	0.26	0.09	6.24

*AUC = area under the plasma drug concentration versus time curve; CL = total clearance; V_{SS} = steady-state volume of distribution; MRT = mean residence time; and $t^{1/2}$ = half-life; F = bioavailability; and K_a = first order absorption rate constant.

TABLE 11

CSF/Serum Ratio of FTC and Its Deaminated Metabolite 1 Hour After Treatment

Monkey	Route	FTC	Metabolite (FTU)
RUh	I.V.	0.076	0.024
RMi	I.V.	0.062	0.032
RJd	I.V.	0.162	0.052
Mean		0.100	0.036
±S.D.		0.054	0.014
RUh	P.O.	0.048	0.026
RMi	P.O.	0.039	0.037
RJd	P.O.	0.117	0.055
Mean		0.068	0.039
±S.D.		0.043	0.015

EXAMPLE 19

CSF/serum Ratio of FTC and its Metabolites in Rhesus Monkeys

The ability of (±)-FTC to cross the blood-brain barrier was evaluated by administering 33.3 mg/kg of the active compound to rhesus monkeys, and measuring the amount of (±)-FTC in the cerebral spinal fluid (CSF) and blood serum one hour after administration. The results are provided in Table 11. The data indicates that a significant amount of active compound passes through the blood-brain barrier in this mammal.

III. Preparation of Pharmaceutical Compositions.

Humans suffering from diseases caused by HIV or HBV infection can be treated by administering to the patient an effective amount of (±)-FTC, or its (−) or (+) enantiomer or a pharmaceutically acceptable derivative or salt thereof in the presence of a pharmaceutically acceptable carrier or diluent. The active materials can be administered by any appropriate route, for example, orally, parenterally, intravenously, intradermally, subcutaneously, or topically, in liquid or solid form.

The active compound is included in the pharmaceutically acceptable carrier or diluent in an amount sufficient to deliver to a patient a therapeutically effective amount of compound to inhibit viral replication in vivo, especially HIV and HBV replication, without causing serious toxic effects in the patient treated. By "inhibitory amount" is meant an amount of active ingredient sufficient to exert an inhibitory effect as measured by, for example, an assay such as the ones described herein.

A preferred dose of (−), (+), or (±)-FTC for all of the above-mentioned conditions will be in the range from about 1 to 50 mg/kg, preferably 1 to 20 mg/kg, of body weight per day, more generally 0.1 to about 100 mg per kilogram body weight of the recipient per day. The effective dosage range of the pharmaceutically acceptable derivatives can be calculated based on the weight of the parent nucleoside to be delivered. If the derivative exhibits activity in itself, the effective dosage can be estimated as above using the weight of the derivative, or by other means known to those skilled in the art.

The compound is conveniently administered in unit any suitable dosage form, including but not limited to one containing 7 to 3000 mg, preferably 70 to 1400 mg of active ingredient per unit dosage form. A oral dosage of 50–1000 mg is usually convenient.

Ideally the active ingredient should be administered to achieve peak plasma concentrations of the active compound of from about 0.2 to 70 μM, preferably about 1.0 to 10 μM. This may be achieved, for example, by the intravenous injection of a 0.1 to 5% solution of the active ingredient, optionally in saline, or administered as a bolus of the active ingredient.

The concentration of active compound in the drug composition will depend on absorption, inactivation, and excretion rates of the drug as well as other factors known to those of skill in the art. It is to be noted that dosage values will also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that the concentration ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition. The active ingredient may be administered at once, or may be divided into a number of smaller doses to be administered at varying intervals of time.

A preferred mode of administration of the active compound is oral. Oral compositions will generally include an inert diluent or an edible carrier. They may be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition.

The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring. When the dosage unit form is a capsule, it can contain, in addition to material of the above type, a liquid carrier such as a fatty oil. In addition, dosage unit forms can contain various other materials which modify the physical form of the dosage unit, for example, coatings of sugar, shellac, or other enteric agents.

(±)-FTC, or its (−) or (+)-enantiomer or pharmaceutically acceptable salts thereof can be administered as a component of an elixir, suspension, syrup, wafer, chewing gum or the like. A syrup may contain, in addition to the active compounds, sucrose as a sweetening agent and certain preservatives, dyes and colorings and flavors.

(±)-FTC, or its (−) or (+)-enantiomers, or pharmaceutically acceptable derivatives or salts thereof can also be mixed with other active materials that do not impair the desired action, or with materials that supplement the desired action, such as antibiotics, antifungals, antiinflammatories, or other antivirals, including other nucleoside anti-HIV compounds.

Solutions or suspensions used for parenteral, intradermal, subcutaneous, or topical application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parental preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

If administered intravenously, preferred carriers are physiological saline or phosphate buffered saline (PBS).

In a preferred embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc.

Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) are also preferred as pharmaceutically acceptable carriers. These may be prepared according to methods known to those skilled in the art, for example, as described in U.S. Pat. No. 4,522,811 (which is incorporated herein by reference in its entirety). For example, liposome formulations may be prepared by dissolving appropriate lipid(s) (such as stearoyl phosphatidyl ethanolamine, stearoyl phosphatidyl choline, arachadoyl phosphatidyl choline, and cholesterol) in an inorganic solvent that is then evaporated, leaving behind a thin film of dried lipid on the surface of the container. An aqueous solution of the active compound or its monophosphate, diphosphate, and/or triphosphate derivatives are then introduced into the container. The container is then swirled by hand to free lipid material from the sides of the container and to disperse lipid aggregates, thereby forming the liposomal suspension.

IV. Preparation of Phosphate Derivatives of FTC

Mono, di, and triphosphate derivative of FTC can be prepared as described below.

The monophosphate can be prepared according to the procedure of Imai et al., *J. Org. Chem.*, 34(6), 1547–1550 (June 1969). For example, about 100 mg of FTC and about 280 μl of phosphoryl chloride are reacted with stirring in about 8 ml of dry ethyl acetate at about 0° C. for about four hours. The reaction is quenched with ice. The aqueous phase is purified on an activated charcoal column, eluting with 5% ammonium hydroxide in a 1:1 mixture of ethanol and water. Evaporation of the eluant gives ammonium FTC-5'-monophosphate.

The diphosphate can be prepared according to the procedure of Davisson et al., *J. Org. Chem.*, 52(9), 1794–1801 (1987). FTC diphosphate can be prepared from the corresponding tosylate, that can be prepared, for example, by reacting the nucleoside with tosyl chloride in pyridine at room temperature for about 24 hours, working up the product in the usual manner (e.g., by washing, drying, and crystallizing it).

The triphosphate can be prepared according to the procedure of Hoard et al., *J. Am. Chem. Soc.*, 87(8), 1785–1788 (1965). For FTC is activated (by making a imidazolide, according to methods known to those skilled in the art) and treating with tributyl ammonium pyrophosphate in DMF. The reaction gives primarily the triphosphate of the nucleoside, with some unreacted monophosphate and some diphosphate. Purification by anion exchange chromatography of a DEAE column is followed by isolation of the triphosphate, e.g., as the tetrasodium salt.

This invention has been described with reference to its preferred embodiments. Variations and modifications of the invention, will be obvious to those skilled in the art from the foregoing detailed description of the invention. It is intended that all of these variations and modifications be included within the scope of the appended claims.

We claim:

1. A derivative of β-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane of the formula:

wherein R_1 is selected from the group consisting of hydrogen, acetyl, propionyl, butyryl, and pentanoyl and R_2 is hydrogen.

2. The mono, di, or trihosphate ester of β-2-Hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane.

3. The compound of claim 1, wherein R_1 is butpryl and R_2 is hydrogen.

4. A physiologically acceptable salt of β-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane.

5. A pharmaceutical composition comprising an effective amount to treat HIV in humans of a physiologically acceptable salt of β-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane.

6. A pharmaceutical composition comprising an effective amount to treat HIV in humans of the mono-, di- or triphosphate ester of β-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane.

7. A pharmaceutical composition comprising an effective amount to treat HIV in humans of the acetyl, propionyl, butyryl, or pentanoyl ester of β-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane.

* * * * *

JUL 9 1998

Della

18085.105096

12/15/98

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Dennis C. Liotta, et al.

Serial No.: 08/488,097 Art Unit: 1611

Filed: June 7, 1995 Examiner: J. Ford

For: Antiviral Activity and Resolution of 2-Hydroxymethyl-5-(5-Fluorocytosin-1-yl)-1,3-Oxathiolane

RECEIVED
JUL 16 1998
GROUP 1800

Assistant Commissioner for Patents
Washington, DC 20231

Terminal Disclaimer

Sir:

Assignee, the owner of entire interest of U.S. Serial No. 08/488,097, through the undersigned attorney of record, hereby disclaims the terminal part of any patent granted on U.S. Serial No. 08/488,097 that would extend beyond the expiration date of the full statutory term of U. S. Serial No. 08/402,730. Any patent granted on U.S. Serial No. 08/488,097 shall be enforceable only for and during such period that legal title to the patent shall be the same as legal title to U. S. Serial No. 08/402,730 and will be binding upon the grantee, its successors or assigns. Dennis C. Liotta, Raymond F. Schinazi and Woo-Baeg Choi have assigned U.S. Serial No. 08/488,097 to Emory University, whose address is of record, in an Assignment recorded on April 10, 1992, commencing at Reel 6128, Frame 0088. U.S. Serial No. 08/402,730 was assigned to Emory University in an Assignment from Dennis C. Liotta, Raymond F. Schinazi and Woo-Baeg Choi recorded on September 18, 1991, commencing at Reel 5876, Frame 0364.

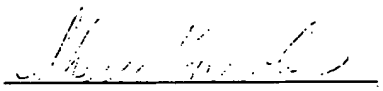
07/15/1998 NHARMOL 00000044 08488497
01 FC:1340 55.00 OP

U.S.S.N. 08/488,097
Filed: June 7, 1995
TERMINAL DISCLAIMER

Assignee does not disclaim any terminal part of any patent granted on U. S. Serial No. 08/488,097 prior to the expiration date of the full statutory term of any patent issuing on U. S. Serial No. 08/402,730 in the event that such patent later: a) expires for failure to pay a maintenance fee, b) is held unenforceable, c) is found invalid, d) is statutorily disclaimed in whole or terminally disclaimed under 37 C.F.R. § 1.321(a), e) has all claims canceled by a reexamination certificate, or f) is otherwise terminated prior to expiration of its statutory term, except for the separation of legal title stated above.

Enclosed is a check in the amount of $55.00 to cover the fee for filing this terminal disclaimer. If this fee is insufficient, please charge Deposit Account No. 11-0980.

Respectfully submitted,



Sherry M. Knowles
Reg. No. 33, 052

KING & SPALDING
191 Peachtree Street
Atlanta, GA 30303
404-572-3541

18085.105096

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Dennis C. Liotta, et al.		
Serial No.:	08/488,097	Art Unit:	1611
Filed:	June 7, 1995	Examiner:	J. Ford

For: Antiviral Activity and Resolution of 2-Hydroxymethyl-5-(5-Fluorocytosin-1-yl)-1,3-Oxathiolane

#20 OK
I.D.
Dee
12/15/98

Assistant Commissioner for Patents
Washington, DC 20231

Terminal Disclaimer

Sir:

Assignee, the owner of entire interest of U.S. Serial No. 08/488,097, through the undersigned attorney of record, hereby disclaims the terminal part of any patent granted on U.S. Serial No. 08/488,097 that would extend beyond the expiration date of the full statutory term of U. S. Serial No. 08/482,875. Any patent granted on U.S. Serial No. 08/488,097 shall be enforceable only for and during such period that legal title to the patent shall be the same as legal title to U. S. Serial No. 08/482,875 and will be binding upon the grantee, its successors or

12/16/1998 CHEIGH 00000002 08488097
01 FC:248 55.00 CH

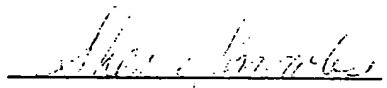
assigns. Dennis C. Liotta, Raymond F. Schinazi and Woo-Baeg Choi have assigned U.S. Serial No. 08/488,097 to Emory University, whose address is of record, in an Assignment recorded on April 10, 1992, commencing at Reel 6128, Frame 0088. U.S. Serial No. 08/482,875 was assigned to Emory University in an Assignment from Dennis C. Liotta, Raymond F. Schinazi and Woo-Baeg Choi recorded on April 10, 1992, commencing at Reel 6128, Frame 0088.

U.S.S.N. 08/488,097
Filed: June 7, 1995
TERMINAL DISCLAIMER

Assignee does not disclaim any terminal part of any patent granted on U. S. Serial No. 08/488,097 prior to the expiration date of the full statutory term of any patent issuing on U. S. Serial No. 08/482,875 in the event that such patent later: a) expires for failure to pay a maintenance fee, b) is held unenforceable, c) is found invalid, d) is statutorily disclaimed in whole or terminally disclaimed under 37 C.F.R. § 1.321(a), e) has all claims canceled by a reexamination certificate, or f) is otherwise terminated prior to expiration of its statutory term, except for the separation of legal title stated above.

Enclosed is a check in the amount of $55.00 to cover the fee for filing this terminal disclaimer. If this fee is insufficient, please charge Deposit Account No. 11-0980.

Respectfully submitted,



Sherry M. Knowles
Reg. No. 33, 052

KING & SPALDING
191 Peachtree Street
Atlanta, GA 30303
404-572-3541

18085.105096

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Dennis C. Liotta, et al.

Serial No.: 08/488,097 Art Unit: 1611

Filed: June 7, 1995 Examiner: J. Ford

For: Antiviral Activity and Resolution of 2-Hydroxymethyl-5-(5-Fluorocytosin-1-yl)-1,3-Oxathiolane

Ok
21/T.D.(3
Dello
12/15/98

Assistant Commissioner for Patents
Washington, DC 20231

Terminal Disclaimer

Sir:

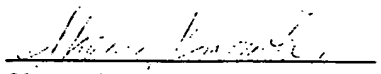
Assignee, the owner of entire interest of U.S. Serial No. 08/488,097, through the undersigned attorney of record, hereby disclaims the terminal part of any patent granted on U.S. Serial No. 08/488,097 that would extend beyond the expiration date of the full statutory term of U. S. Serial No. 08/474,406. Any patent granted on U.S. Serial No. 08/488,097 shall be enforceable only for and during such period that legal title to the patent shall be the same as legal title to U. S. Serial No. 08/474,406 and will be binding upon the grantee, its successors or assigns. Dennis C. Liotta, Raymond F. Schinazi and Woo-Baeg Choi have assigned U.S. Serial No. 08/488,097 to Emory University, whose address is of record, in an Assignment recorded on April 10, 1992, commencing at Reel 6128, Frame 0088. U.S. Serial No. 08/474,406 was assigned to Emory University in an Assignment from Dennis C. Liotta, Raymond F. Schinazi and Woo-Baeg Choi recorded on September 18, 1991, commencing at Reel 5876, Frames 0364-0368.

U.S.S.N. 08/488,097
Filed: June 7, 1995
TERMINAL DISCLAIMER

Assignee does not disclaim any terminal part of any patent granted on U. S. Serial No. 08/488,097 prior to the expiration date of the full statutory term of any patent issuing on U. S. Serial No. 08/474,406 in the event that such patent later: a) expires for failure to pay a maintenance fee, b) is held unenforceable, c) is found invalid, d) is statutorily disclaimed in whole or terminally disclaimed under 37 C.F.R. § 1.321(a), e) has all claims canceled by a reexamination certificate, or f) is otherwise terminated prior to expiration of its statutory term, except for the separation of legal title stated above.

Enclosed is a check in the amount of $55.00 to cover the fee for filing this terminal disclaimer. If this fee is insufficient, please charge Deposit Account No. 11-0980.

Respectfully submitted,



Sherry M. Knowles
Reg. No. 33, 052

KING & SPALDING
191 Peachtree Street
Atlanta, GA 30303
404-572-3541

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Dennis C. Liotta, et al.

Serial No.: 08/488,097 Art Unit: 1623

Filed: June 7, 1995 Examiner: Wilson

For: Antiviral Activity and Resolution of 2-Hydroxymethyl-5-(5-Fluorocytosin-1-yl)-1,3-Oxathiolane

OFFICIAL

Assistant Commissioner for Patents
Washington, DC 20231

Terminal Disclaimer

Sir:

Assignee, the owner of entire interest of U.S. Serial No. 08/488,097, through the undersigned attorney of record, hereby disclaims the terminal part of any patent granted on U.S. Serial No. 08/488,097 that would extend beyond the expiration date of the full statutory term of U. S. Patent No. 5,814,639. Any patent granted on U.S. Serial No. 08/488,097 shall be enforceable only for and during such period that legal title to the patent shall be the same as legal title to U. S. Patent No. 5,814,639 and will be binding upon the grantee, its successors or assigns. Dennis C. Liotta, Raymond F. Schinazi and Woo-Baeg Choi have assigned U.S. Serial No. 08/488,097 to Emory University, whose address is of record, in an Assignment recorded on April 10, 1992, commencing at Reel 6128, Frame 0088. U.S. Patent No. 5,814,639 was assigned to Emory University in an Assignment from Dennis C. Liotta, Raymond F. Schinazi and Woo-Baeg Choi recorded on November 20, 1991, commencing at Reel 5616, Frame 0588.

U.S.S.N. 08/488,097
Filed: June 7, 1995
TERMINAL DISCLAIMER

Assignee does not disclaim any terminal part of any patent granted on U. S. Serial No. 08/488,097 prior to the expiration date of the full statutory term of U. S. Patent No. 5,814,639 in the event that such patent later: a) expires for failure to pay a maintenance fee, b) is held unenforceable, c) is found invalid, d) is statutorily disclaimed in whole or terminally disclaimed under 37 C.F.R. § 1.321(a), e) has all claims canceled by a reexamination certificate, or f) is otherwise terminated prior to expiration of its statutory term, except for the separation of legal title stated above.

Enclosed is a check in the amount of $55.00 to cover the fee for filing this terminal disclaimer. If this fee is insufficient, please charge Deposit Account No. 11-0980.

Respectfully submitted,

Sherry M. Knowles
Reg. No. 33, 052

KING & SPALDING
191 Peachtree Street
Atlanta, GA 30303
404-572-3541 (Telephone)
404-572-5145 (Facsimile)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor: Dennis C. Liotta, et al.

Serial No.: 08/488,097 U.S. Patent No.: 5,914,331

Filed: June 7, 1995 Issued: June 22, 1999

Title: Antiviral Activity and Resolution of 2-Hydroxymethyl-5-(5-Fluorocytosin-1-yl)-1,3-Oxathiolane

Certificate of Correction Branch
Commissioner for Patents
Washington, D.C. 20231

REQUEST FOR CERTIFICATE OF CORRECTION FOR PATENT OFFICE'S MISTAKE (37 C.F.R. 1.322)

Sir:

Patentee notes that errors of a typographical nature or character appear in the patent as a result of Patent and Trademark Office (PTO) mistakes. The attached Form PTO/SB/44, in duplicate, describes the errors in detail. Patentee respectfully requests that the Commissioner issue a Certificate of Correction to correct the errors appearing in the printed patent due to PTO mistakes. Correction thereof does not involve such changes in the patent as would constitute new matter or would require re-examination.

Patentee submits that no fee is due with the filing of this paper. However, the Commissioner is hereby authorized to charge any necessary fee to Deposit Account No. 11-0980. A duplicate of this paper is attached.

Respectfully submitted,

Sherry M. Knowles, Esq.
Registration No. 33,052
Customer No. 20786

KING & SPALDING
191 Peachtree Street
Atlanta, Georgia 30303-1763
(404) 572-3541 (Telephone)
(404) 572-5145 (Facsimile)
K&S Docket No. 18085.105096

CERTIFICATE OF MAILING

I hereby certify that this Request for Certificate of Correction, along with any documents indicated as being attached, is being deposited on the date shown below with the United States Postal Service as first class mail in an envelope addressed to Certificate of Correction Branch, Commissioner for Patents, Washington, D.C. 20231.

William O. Isaacs, II, Reg No. 44,165

Date: 11/5/02

PTO/SB/44 (02-01)
Approved for use through 01/31/2004. OMB 0651-0033
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.
(Also Form PTO-1050)

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO : 5,914,331

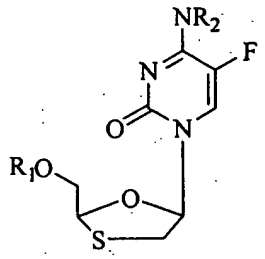
DATED : June 22, 1999

INVENTOR(S) : Dennis C. Liotta, et al

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In Claim 1, Column 30, Line 37, the formula has been omitted and should read

--



--

In Claim 2, Column 30, Line 41, "trihosphate" should read --triphosphate--.

In Claim 3, Column 30, Line 43, "butpryl" should read --butyryl--.

MAILING ADDRESS OF SENDER:
Sherry M. Knowles, Esq.
King & Spalding
191 Peachtree Street
Atlanta, GA 30303-1763

PATENT NO. 5,914,331

No. of additional copies

⇒ 0

PTO/SB/44 (02-01)
Approved for use through 01/31/2004. OMB 0651-0033
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.
(Also Form PTO-1050)

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO : 5,914,331

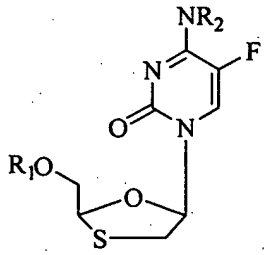
DATED : June 22, 1999

INVENTOR(S) : Dennis C. Liotta, et al

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In Claim 1, Column 30, Line 37, the formula has been omitted and should read

--



--

In Claim 2, Column 30, Line 41, "trihosphate" should read --triphosphate--.

In Claim 3, Column 30, Line 43, "butpryl" should read --butyryl--.

MAILING ADDRESS OF SENDER:
Sherry M. Knowles, Esq.
King & Spalding
191 Peachtree Street
Atlanta, GA 30303-1763

PATENT NO. 5,914,331

No. of additional copies

⇒ 0

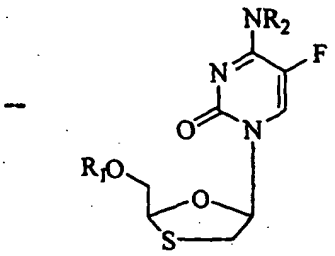
UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,914,331
DATED : June 22, 1999
INVENTOR(S) : Dennis C. Liotta et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 30,
Line 37, the formula has been omitted and should read



Column 30,
Line 41, "trihosphate" should read -- triphosphate --.
Line 43, "butpryl" should read -- butyryl --.

Signed and Sealed this

Eleventh Day of March, 2003

JAMES E. ROGAN
Director of the United States Patent and Trademark Office

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

000000

MFFC

SHERRY M. KNOWLES, ESQ
KING & SPALDING
191 PEACHTREE STREET
ATLANTA GA 30303-1763

MAINTENANCE FEE STATEMENT

The data shown below is from the records of the Patent and Trademark Office. If the maintenance fees and any necessary surcharges have been timely paid for the patents listed below, the notation "PAID" will appear in column 11, "STAT" below.

If a maintenance fee payment is defective, the reason is indicated by code in column 11, "STAT" below. TIMELY CORRECTION IS REQUIRED IN ORDER TO AVOID EXPIRATION OF THE PATENT. NOTE 37 CFR 1.377. THE PAYMENT(S) WILL BE ENTERED UPON RECEIPT OF ACCEPTABLE CORRECTION. IF PAYMENT OR CORRECTION IS SUBMITTED DURING THE GRACE PERIOD, A SURCHARGE IS ALSO REQUIRED. NOTE 37 CFR 1.20(h).

If the statement of small entity status is defective the reason is indicated below in column 10 for the related patent number. THE STATEMENT OF SMALL ENTITY STATUS WILL BE ENTERED UPON RECEIPT OF ACCEPTABLE CORRECTION.

ITEM NBR	PATENT NUMBER	FEE CDE	FEE AMT	SUR CHARGE	APPL NUMBER	PATENT DATE	FILE DATE	PAY YR	SML ENT	STAT
1	5,914,331	1551	880	0	08/488,097	06/22/99	06/07/95	04	NO	PAID

ITM NBR	ATTY DKT NUMBER
1	

DIRECT THE RESPONSE, TOGETHER WITH ANY QUESTIONS ABOUT THIS NOTICE TO: COMMISSIONER OF PATENTS AND TRADEMARKS, BOX M CORRESPONDENCE, WASHINGTON, D.C. 20231

EXHIBIT G
DESCRIPTION OF SIGNIFICANT ACTIVITIES FOR EMTRIVA (EMTRICITABINE) CAPSULES IND 53,971 AND NDA 21-500

The following is a description of significant activities during the regulatory review period for emtricitabine capsules, which was also known as FTC. The description includes interactions with FDA by Gilead Sciences and by Triangle Pharmaceuticals, Inc. (TPI), the company which originally submitted IND 53,971 and NDA 21-500. Gilead Sciences acquired Triangle Pharmaceuticals, Inc. in January 2003, and acquired the IND and NDA as well. In addition to the listed activities, TPI and Gilead Sciences also submitted to FDA additional material, including reports of adverse events in individual patients, and had additional communications relating to such materials.

Date of Submission to FDA or other Communication with FDA	**Description**
8/20/97	Original IND Submission
9/9/97	Fax Response to FDA request for clinical trial material labeling
9/17/97	Fax from FDA with comments directed towards IND
9/17/97	Fax from FDA with comments directed towards IND
9/20/97	Effective date of IND
9/24/97	Submitted Amendments 1 and 2, Protocol FTC-101
10/1/97	Submitted Amendment 3, Protocol FTC-101
10/20/97	Fax from FDA with comments directed towards IND
10/21/97	Submitted Amendment 4, Protocol FTC-101
11/14/97	Submitted New Investigators for FTC-101
12/9/97	Submitted New Investigators for FTC-101
1/22/98	Submitted abstract and press release
1/28/98	Telephone conference with FDA re: FDA comments on press release
2/3/98	Submitted Amendment 6, Protocol FTC-101
2/24/98	Submitted New Investigators for FTC-101
2/25/98	Submitted Protocol FTC-103 and Amendment 1, and investigators
4/2/98	Submitted Protocol FTC-104 and Amendment 1
4/15/98	Submitted Revised 1572s for previously registered investigators
6/2/98	Submitted CMC Data & Response to FDA letters of 9/17/97 and 10/20/97
7/13/98	Submitted Request for Fast Track Status
8/6/98	Submitted Follow-Up Letter to Request for Fast Track Status
8/24/98	Letter to FDA Requesting an End of Phase 2 Meeting and Submitting proposed Protocol FTC-303
9/2/98	Submitted Protocol FTC-102 incorporating Amendments 1-4 and New Investigators
9/22/98	Fax from FDA re: comments relating to FTC-102

Date of Submission to FDA or other Communication with FDA	Description
9/24/98	Submitted Protocol FTC-303, Amendment 1 to it and New Investigators; and Submitted Manufacturing and Controls Update
9/25/98	Letter from FDA re: Fast Track Status
10/1/98	Submitted New Investigators for FTC-303
10/9/98	Submitted New Investigators for FTC-303
10/12/98	Submitted New Investigators for FTC-303
10/15/98	Submitted End-of-Phase 2 Meeting Data Package
10/22/98	Submitted New Investigator for FTC-303
10/28/98	Meeting with FDA on Clinical Development Plans
11/10/98	Submitted New Investigator for FTC-303
11/13/98	Fax from FDA re: clinical comments for Protocol FTC-303
11/20/98	Submitted IND Annual Report for period covering August 1997 through August 1998
11/20/98	Submitted New Investigators for FTC-303, revised 1572s for investigators, and replacement of primary investigator
11/24/98	Submitted preclinical reports: Dose Range-Finding Study in Rabbits, Dose Range-Finding Study in Mice, and Six Month Oral Tox in Mice
12/1/98	Fax from FDA re: statistical comments on 9/24/98 submission
12/10/98	Submitted New Investigators for FTC-303 and revised 1572s for investigators
12/14/98	Submitted New Investigators for FTC-303
12/15/98	Submitted revised 1572s for FTC-303
12/28/98	Submitted New Investigators for FTC-303 and revised 1572s for investigators
1/11/99	Fax from FDA re: Chemistry comments
1/11/99	Submitted New Investigators for FTC-303 and revised 1572s for investigators
1/18/99	Submitted Follow-Up to FDA Meeting of 10/28/98
1/18/99	Submitted New Investigator for FTC-303 and revised 1572s
1/26/99	Submitted preclinical reports: Six Month Oral in Mice w/ 3-Month Interim Kill, Effects of FTC on Embryo/Fetal Development in Mice, and Toxicokinetic Effects of FTC on Embryo/Fetal Development in Mice
2/1/99	Submitted New Investigator for FTC-303 and revised 1572s for investigators
2/2/99	E-mail from FDA re: requesting more safety data from FTC-101 and FTC-102
2/3/99	Teleconference with FDA re: FTC-302
2/10/99	Submitted Safety Data for FTC-101 and FTC-102
2/15/99	Submitted New Investigators for FTC-303

Date of Submission to FDA or other Communication with FDA	Description
3/12/99	Submitted revised 1572s for FTC-303
3/18/99	Submitted Draft Protocol FTC-302 and Request for End-of-Phase 2 Meeting
3/23/99	Submitted Request to FDA for Review of Trade Name
4/7/99	Fax from FDA re: clinical comments on Draft Protocol FTC-302
4/12/99	Submitted preclinical reports: Study of Fertility and Early Embryonic Development in CD-1 Mice and Pre and Postnatal Development in CD-1 Mice
4/14/99	Submitted New Protocol FTC-105 and New Investigator with CMC Data for the study
4/20/99	Submitted Amendment 3, Protocol FTC-303
4/22/99	Submitted CMC Amendment for new synthesis and controls
4/30/99	Teleconference with FDA re: biopharmaceutical comments on FTC-105
5/6/99	Submitted Response to FDA Fax of 4/7/99 for additional information and revised Protocol FTC-302
5/11/99	Submitted New Investigator for FTC-105 and revised 1572s for FTC-303
5/14/99	Submitted New Investigator for FTC-105 and revised 1572 for FTC-303
5/26/99	Call from FDA re: notice that FDA will send comments on FTC Protocol FTC-302
5/27/99	Submitted New Protocol FTC-301
6/7/99	Fax from FDA re: clinical comments relating to 5/6/99 submission
6/10/99	Teleconference with FDA re: Protocol FTC-302 and potential meeting
6/16/99	Response to FDA Fax of 5/21/99 containing clinical comments re: FTC-105
6/17/99	Submitted New Investigators for FTC-105 and revised 1572s for FTC-303
6/24/99	Submitted New Investigator for FTC-105
7/15/99	Letter from FDA re: Clinical and Statistical comments on FTC-301
7/27/99	Submitted New Investigator for FTC-301 and revised 1572s for FTC-303
7/27/99	Submitted CMC Data to Support Overencapsulated Clinical Trial Material
8/3/99	Submitted New Investigators for FTC-302 and FTC-105
8/16/99	Submitted New Investigator for FTC-301 and revised 1572s for FTC-303
8/18/99	Submitted Response to FDA comments on 6/7/99 and Amendment 1, Protocol FTC-302
8/20/99	Submitted New Investigators for FTC-301 and FTC-302
8/26/99	Submitted Information Amendment re: Amendment 5 to FTC-101, Amendments 2 and 4 to FTC-303, New Investigators for FTC-302 and revised 1572 for FTC-303
8/31/99	Submitted New Protocol FTC-112, New Investigators for FTC-301, revised 1572s for FTC-303 and FTC-105

Date of Submission to FDA or other Communication with FDA	Description
9/2/99	Submitted NDA Plan and Response to FDA comments on FTC-301 and FTC-302
9/10/99	Submitted FTC Protocol FTC-350 and New Investigator for FTC-302
9/17/99	Letter from FDA re: conducting studies in treatment-experienced patients
9/20/99	Submitted Amendment 2, Protocol FTC-302 and New Investigators for FTC-112, FTC-301, FTC-302, and FTC-350
9/22/99	Fax from FDA re: comments relating to FTC-112
10/1/99	Submitted New Investigators for FTC-301 and FTC-350 and revised 1572 for FTC-303
10/7/99	Submitted New Investigators for FTC-350
10/8/99	Submitted New Protocol FTC-106 and New Investigator and submitted CMC Data and Certificate of Analysis for 14C Radiolabeled Emtricitabine Study
10/13/99	Fax from FDA re: comments on FTC-350
10/21/99	Submitted New Investigators for FTC-350
11/3/99	Submitted Response to FDA fax dated 10/13/99 re: FTC-350
11/4/99	Submitted New Protocol FTC-107 and Investigator and New Investigators for FTC-301 and FTC-350
11/4/99	Letter from FDA providing comments on clinical development and NDA Plan
11/18/99	Submitted IND Annual Report for period covering September 1998 through August 1999
11/23/99	Submitted One Year Monkey Study
11/29/99	Submitted Response to FDA Letter of 11/4/99 re: Clinical Development Plan for FTC
11/30/99	Submitted preclinical reports: An Acute Oral Tox in Mice, Acute Intravenous Tox in Mice, Acute Oral Tox in Rats, and DRAFT report – Fertility Study in Male Rats Given 524W91
12/2/99	Submitted New Investigators for FTC-301 and FTC-350, and revised 1572s for FTC-303 and FTC-105
12/17/99	Submitted CMC Amendment re: new formulation and manufacturing sites
12/17/99	Submitted revised protocol synopsis for Protocol FTC-301 in response to FDA correspondence dated 7/15/99 and 11/4/99
12/28/99	Telephone conference with FDA re: changes in conduct of FTC-302
12/28/99	Response to FDA request for additional safety information
12/28/99	Fax from FDA re: agreements for changes in conduct of study for FTC-302 and reporting serious adverse events (SAEs) in clinical trials
1/3/00	Fax to FDA attaching summary of safety data from study FTC-302
1/4/00	Submitted New Investigators for FTC-301 and FTC-350

Date of Submission to FDA or other Communication with FDA	Description
1/4/00	Fax to FDA attaching preliminary list of SAEs in FTC-302 and FTC-303
1/5/00	FDA request for meeting to discuss whether to continue the FTC-302 trial
1/10/00	Submitted information in response to FDA fax of 12/28/99 for FTC-302
1/11/00	Submitted FDA Meeting Package for 1/12/00 meeting to discuss FTC-302
1/11/00	Submitted summaries of SAE reports for FTC studies requested by FDA
1/12/00	Meeting with FDA on Safety Reporting
1/17/00	Submitted Responses to FDA from 1/12/00 Meeting
1/17/00	Submitted additional SAE information as agreed in FDA fax dated 12/28/99
1/20/00	Telephone call with FDA re: FDA request for list of South African investigators and what should be addressed with clinical steering committee on FTC-302
1/20/00	Fax from FDA re: Clinical comments on FTC-301
1/21/00	Submitted New Protocol FTC-109 and New Investigator
1/21/00	Fax from FDA re: Clinical comments on FTC-301
1/22/00	Fax to FDA attaching list of investigators in FTC-302
1/24/00	Submitted New Investigators for FTC-105 and FTC-350, and revised 1572s for FTC-303 and FTC-105
1/31/00	Fax from FDA providing summary of recommendations and agreements from 1/12/00 meeting
2/2/00	Submitted Summary of Actions for FTC-302
2/2/00	Submitted safety data
2/3/00	Teleconference with FDA about discussion with Medicines Control Council (MCC) of South Africa about FTC-302
2/7/00	Fax from FDA providing comments relating to the SOP submitted 1/17/00
2/11/00	Submitted New Investigators for FTC-350
2/11/00	Submitted CMC Amendment adding alternate manufacturer of over-encapsulated clinical trial material
2/16/00	Submitted New Protocol FTC-108 and principal investigator
2/16/00	Submitted safety data
2/18/00	Submitted to FDA Copies of all MCC Communications on FTC-302
2/18/00	Response to FDA Fax of 1/20/00 re: Protocol FTC-301
3/1/00	Submitted New Investigators for FTC-303 and FTC-350 and revised 1572s for FTC-303
3/1/00	Submitted Minutes of the Clinical Steering Committee for FTC-302
3/1/00	Call with FDA re: FTC-108
3/3/00	Submitted safety data
3/8/00	Fax to FDA re: e-mail from MCC on resolution and recommendation on FTC-302

Date of Submission to FDA or other Communication with FDA	Description
3/13/00	Submitted Protocol FTC-301
3/14/00	Submitted New Investigators for FTC-105 and FTC-350 and revised 1572 for FTC-303
3/17/00	Submitted safety data
3/20/00	Submitted Revised Amendment 3, FTC-302 with MCC Changes
3/20/00	Submitted Amendment 1, Protocol FTC-301 and version incorporating Amendments 1 and 2
3/21/00	Submitted FTC preclinical report: Tissue Distribution and Excretion Study in Rats
3/21/00	Submitted CMC amendment providing modifications to synthesis of drug substance and for a manufacturing site for 100 mg capsules
3/28/00	Submitted New Investigators for FTC-350 and revised 1572 for FTC-303
3/29/00	Letter from FDA outlining requirements of FTC-302 to be considered as principal clinical trial in NDA
3/31/00	Submitted safety data
3/31/00	Submitted to FDA Copy of FTC Waivers for Protocol FTC-302 as submitted to MCC on 3/31/00
4/3/00	Submitted Amendment 4, Protocol FTC-302 and New Investigators for FTC-350
4/5/00	Fax to FDA attaching requested list of investigators, patients screened and enrolled per site, dates of monitoring visits for particular sites and address of sites for FTC-302
4/5/00	Fax to FDA attaching requested table of FTC-302 investigators, number screened, enrolled, waivers granted, safety information and copy of MCC communication
4/6/00	Teleconference with FDA re: FTC-302
4/6/00	Fax to FDA attaching letter from MCC re: termination of FTC-302
4/6/00	Fax to FDA including two press releases issued re: FTC-302 MCC termination of study
4/6/00	Fax to FDA asking for comments on draft press release re: FDA clinical hold of FTC-302
4/12/00	Letter from FDA re: clinical hold for FTC-302
4/12/00	Fax from FDA re: clinical comments on FTC-301
4/14/00	Submitted safety data
4/17/00	Submitted CMC Proposal to FDA for Dissolution Testing
4/17/00	Submitted to FDA Copies of MCC's (South Africa) Resolutions re: FTC-302
4/18/00	Letter of cross-reference authorizing DAIDS to use IND for their Protocol A5015

Date of Submission to FDA or other Communication with FDA	Description
4/20/00	Submitted revised 1572s for FTC-303
4/28/00	Submitted safety data
5/3/00	Fax to FDA attaching letter to MCC with proposal for FTC-302 and press release re: termination
5/12/00	Submitted safety data
5/12/00	Fax from FDA providing comments on CMC Proposal for Dissolution Testing
5/16/00	Fax to FDA attaching correspondence from MCC dated 5/10/00 re: FTC-302 and response dated 5/12/00
5/23/00	Submitted New Investigator for FTC-350 and revised 1572s for FTC-303 and FTC-350
5/26/00	Submitted Response to FDA Letter of 4/12/00 re: FTC-301, Amendment 3, Protocol FTC-301, and Amendment 5, FTC-303, and Statistical Analysis Plan for FTC-303
5/26/00	Submitted safety data
6/12/00	Submitted safety data
6/16/00	Submitted New Protocol FTC-350, Amendment 1 to it, and revised 1572s for FTC-303
6/16/00	Submitted Amendment 1, Protocol FTC-105
7/5/00	Fax from FDA providing clinical comments on 5/26/00
7/6/00	Submitted CMC Amendment for FTC for overencapsulated clinical trial material
7/7/00	Submitted preclinical report: 52-Week Oral Toxicity Study in Cynomolgus Monkeys with 4-Week Recovery Period - Report for Interim Sacrifice at Week 14
7/7/00	Submitted safety data
7/7/00	Fax from FDA providing comments on Amendment 1, FTC-105
7/11/00	Submitted CMC Amendment concerning the manufacturing site for the drug substance, supplier of an intermediate, and use of Blue/White Capsules for the drug product
8/1/00	Submitted New Investigators for FTC-301 and revised 1572s for FTC-301 and FTC-303
8/4/00	Teleconference with FDA acknowledging revised format for FTC safety update is acceptable
8/8/00	Submitted clinical study report for FTC-112
8/8/00	Submitted to FDA copy of MCC Resolution of 8/7/00 re: FTC-302
8/8/00	Submitted safety data
8/11/00	Submitted New Investigators for FTC-301
8/11/00	Fax to FDA attaching TPI correspondence with the MCC (South Africa)

Date of Submission to FDA or other Communication with FDA	Description
8/14/00	Fax to FDA attaching current synopsis for FTC-301 and dosing of clinical trial material
8/20/00	Submitted final clinical study report for FTC-104
8/20/00	Submitted updated Investigators Brochure as of August 2000
8/21/00	Letter to FDA on how TPI decided to proceed with study FTC-301
8/29/00	Submitted New Protocol FTC-113 and New Investigator, New Investigators for FTC-301, and revised 1572s for FTC-105 and FTC-350
9/13/00	Submitted Amendment 4, Protocol FTC-301
9/14/00	Submitted safety data
9/21/00	Fax to FDA attaching abstract presented at 5th Int'l Congress on Drug Therapy in HIV Infection
9/27/00	Submitted working protocol for protocol FTC-301 incorporating Amendments 1-4, New Investigators for FTC-301, revised 1572s for FTC-303 and FTC-350
10/12/00	Submitted preclinical report: 14-Day Oral Bridging Toxicity Study of FTC in Mice
10/13/00	Submitted FTC Protocol FTC-302A to FDA including unblinding of patients in South Africa in FTC-302 and continuing compassionate use
10/17/00	Submitted monthly safety update as of 10/17/00 for FTC including MKC-401
10/20/00	Submitted New Investigators for FTC-105 and FTC-301
10/25/00	Submitted FTC Clinical Steering Committee Minutes to FDA as follow up to monthly safety update dated 10/17/00
10/26/00	Submitted Amendment 1, Protocol FTC-203 and New Investigators for FTC-301
11/9/00	Submitted New Investigators for FTC-301
11/10/00	Submitted Monthly Safety Update as of 11/7/00
11/20/00	Submitted full copy of presentation at Glasgow meeting for FTC-302
11/27/00	Submitted New Investigators for FTC-301
11/28/00	Submitted preclinical reports: TOX022 Six Month Oral Tox Study in Mice, TOX038 Embryo/Fetal Development in Rabbits and TOX103 Fetal Exposures in CD-1 mice
12/8/00	Submitted New Investigators for FTC-301
12/19/00	Submitted CMC Amendment for FTC re: general update to stability for drug substance and drug product and update to method of manufacture
12/20/00	Submitted safety data
1/10/01	Submitted New Investigators for FTC-301
1/10/01	Submitted IND Annual Report for period covering September 1999 through August 2000

Date of Submission to FDA or other Communication with FDA	Description
1/15/01	Submitted Response to FDA Letters of 3/29/00 and 4/12/00 re: clinical hold for FTC-302
1/23/01	Submitted safety data
1/29/01	Submitted New Investigators for FTC-203 and FTC-301
1/30/01	Submitted New Protocol FTC-111 and New Investigator
1/30/01	Submitted preclinical reports: 2 Carcinobioassay Protocols (Mice & Rats) for FTC, Toxicokinetic 3-Month Dose Range Finding Study in Rats, and 3-Month Dose Range Finding Study in Rats (MPI Research Report)
1/31/01	Submitted copies of Retrovirus Presentations on FTC
2/2/01	Faxed to FDA revised pages to Retrovirus Presentations on FTC
2/6/01	Submitted protocol FTC-110 and CMC data for clinical material for protocol
2/9/01	Submitted final clinical study report for FTC-109
2/14/01	Letter from FDA in response to 1/15/01 submission re: FTC-302
2/15/01	Submitted New Investigators for FTC-110, FTC-203 and FTC-301
2/20/01	Submitted Amendment 5, Protocol FTC-301
2/26/01	Submitted Final Clinical Study Report for FTC-103
3/8/01	Submitted Meeting Request to FDA for Informal Meeting
3/12/01	Submitted New Investigators for FTC-301
3/15/01	Fax from FDA re: providing comments on Rat and Mouse Dose Selection Studies
3/21/01	Submitted Clinical Steering Committee Minutes
3/21/01	Fax to FDA attaching South African treatment program for FTC-302
3/23/01	Teleconference with FDA re: safety data
3/30/01	Submitted Request for Clinical Development Meeting
3/30/01	Submitted Meeting Data Package for 4/18/01 FDA Meeting re: FTC-302
4/11/01	Submitted preclinical reports: 3-Month in Rats, 1-Month Mouse, Pharmacokinetics, Excretion and Tissue in C14 in Male Cynomolgus Monkeys, and Metabolism and Excretion of C14 Male Cynomolgus Monkeys
4/16/01	Submitted New Investigators for FTC-203 and FTC-301
4/18/01	Meeting with FDA
4/26/01	Submitted Letter to FDA re: FTC-301 and the differences between FTC-301 and FTC-301A

Date of Submission to FDA or other Communication with FDA	Description
5/7/01	Submitted Amendment 2, Protocol FTC-203 and New Investigator for FTC-301
5/10/01	Submitted final Clinical Steering Committee Meeting Minutes
5/10/01	Submitted to FDA CMC Meeting Minutes from 4/18/01 meeting with FDA and Summary of Waivers for FTC protocol FTC-302
5/15/01	Submitted revised 1572s for FTC-105, FTC-110, FTC-203, FTC-301, FTC-302, FTC-303, and FTC-350
5/16/01	Letter of cross-reference to IND for DAIDS to refer to the IND to support Protocol P1021
5/30/01	Submitted Amendment 6, Protocol FTC-301
6/4/01	Fax to FDA attaching materials relating to the 6/5/01 meeting
6/5/01	Meeting with FDA
6/6/01	Fax from FDA providing comments on 5/30/01 submission
6/7/01	Letter to FDA Submitting copy of MCC Letter Dated 5/18/01 re: FTC-302
6/8/01	Submitted New Investigators for FTC-301 and revised 1572s for FTC-350
6/14/01	Submitted to FDA Proposal of Data and Timelines for FTC NDA as requested at 6/5/01 meeting with FDA and rationale for accelerated approval
6/19/01	Submitted Response to FDA Fax of 6/6/01 in reference to Amendment 6, Protocol FTC-301 re: Definition of Virological Failure in FTC-301
6/28/01	Submitted to FDA Minutes of Meeting Held on 6/5/01
6/29/01	Submitted CMC Briefing Document
7/13/01	Submitted Briefing Paper re: FTC-302
7/19/01	Fax from FDA providing comments on 6/29/01 and 7/13/01 submissions
7/26/01	Submitted New Investigators for FTC-301 and revised 1572 for FTC-350
8/6/01	Submitted FTC CMC update for manufacturing site change for overencapsulated clinical trial material
8/7/01	Submitted to FDA copies of MCC Letter Dated 8/1/01 re: Protocol FTC-302 and Press Release dated 8/6/01
8/9/01	Submitted New Investigator for FTC-301 and revised 1572s for FTC-303 and FTC-350
9/5/01	Fax from FDA providing comments on CMC Briefing Document
10/24/01	Letter to FDA Proposing New NDA Timelines to include safety and efficacy data from FTC-301
11/1/01	Telephone contact with FDA regarding study FTC-302
11/12/01	Submitted New Investigators for FTC-203, FTC-301, and FTC-350
11/16/01	Call with FDA re: review of NDA proposal

Date of Submission to FDA or other Communication with FDA	Description
11/21/01	Submitted to FDA amendment to Investigators Brochure
11/28/01	Fax from FDA requesting List of All Non-Clinical Data to be included in the NDA
11/28/01	Submitted Response to FDA Request for List of All Non-Clinical Data to be included in the NDA
12/5/01	Teleconference with FDA advising that FTC NDA package is acceptable to file in September 2002 with FTC-303 at 48 weeks, FTC-301 at 24 weeks, and 48 week data from FTC-301 filed by March 2003
1/14/02	Teleconference with FDA about wording for press release
1/24/02	Submitted Amendment 3, Protocol FTC-203 and New Investigators, and revised 1572 for FTC-350
1/22/02	Fax from FDA re: request for update on dissolution methodology for formulation
2/4/02	Response to FDA Fax of 1/22/02 requesting update of dissolution methodology for formulations
2/13/02	Letter to FDA providing NDA Clinical Report Content Proposal and requesting guidance
2/20/02	Submitted IND Annual Report for period covering September 2000 through August 2001
2/26/02	Submitted New Investigators for FTC-203, and revised 1572s for FTC-301 and FTC-350
3/6/02	Submitted to FDA amendments to toxicology reports
3/21/02	Request for Small Business Waiver of User Fee for FTC NDA
3/28/02	Response to FDA fax dated 9/5/01 with responses and questions regarding CMC briefing document
4/1/02	Call with FDA re: NDA plan in 2/13/02 letter and submitting the NDA for traditional review
4/4/02	Letter from FDA re: status of FTC-302
4/15/02	Submitted to FDA letter of cross-reference authorizing DAIDS to reference IND for Protocol A5073
4/18/02	Submitted to FDA New Investigators for FTC-203 and FTC-301 and revised 1572 for FTC-301
5/1/02	Letter to FDA Notifying of Termination of a site for FTC-301
5/2/02	Submitted Letter to FDA with NDA Format Questions
5/3/02	Submitted updated Investigators Brochure dated 5/2/02
5/8/02	Letter from FDA granting request for Small Business Waiver for NDA (Waiver Request No. 2002-020)
5/31/02	Letter to FDA with Pre-NDA Data Package FTC

Date of Submission to FDA or other Communication with FDA	Description
6/6/02	E-mail to FDA requesting teleconference prior to 7/3/02 meeting highlighting results of FTC-301A
6/10/02	Submitted to FDA Clinical Trial Analysis Plan for FTC-301A
6/13/02	Submitted Amendment 7, Protocol FTC-301
6/13/02	Submitted to FDA CRO responsibilities for FTC Protocols FTC-301, FTC-302, and FTC-303
6/21/02	Fax to FDA providing data results for 6/21/02 conference call re: FTC-301A
6/21/02	Teleconference re: FTC-301A study results
6/25/02	E-mail from FDA requesting preliminary data on ALIZE & ANRS 099
6/25/02	Teleconference with FDA re: FDA recommendations for FTC-301A DSMB
6/25/02	Fax from FDA providing Chemistry comments on 3/28/02 submission
6/25/02	E-mailed to FDA DSMB Charter for FTC-301A and last communication with Data Safety Monitoring Board (DSMB)
6/26/02	E-mail communications with FDA re: data package for DSMB and unblinding FTC-301A
6/26/02	Call with FDA re: DSMB and unblinding of FTC-301A
6/26/02	E-mail communications with FDA re: questions in pre-NDA package, including questions based on FTC-301A results posed during 6/21/02 Teleconference
7/1/02	E-mail from FDA requesting update on feedback from DSMB
7/1/02	E-mail to FDA with update on DSMB response to data package
7/2/02	Teleconference with FDA re: what should be presented at pre-NDA meeting
7/3/02	Pre-NDA Meeting
7/10/02	E-mail to FDA with update re: DSMB recommendation to unblind FTC-301A
7/17/02	E-mail to FDA providing FTC-301A blinded data analysis sent to DSMB according to new algorithm received at Pre-NDA meeting
7/22/02	Pre-Submission of NDA Items 5 Nonclinical Pharmacology & Toxicology and 7 Clinical Microbiology (NDA Volumes 1.1 – 1.5)
7/23/02	Submitted New Investigators for FTC-301
7/23/02	E-mail to FDA re: letters to be sent to investigators on FTC-301
7/23/02	E-mail to FDA attaching patient information and informed consent for FTC-301 patients
7/25/02	E-mail and fax to FDA of revised Informed Consent for FTC-301 and draft letter from sites to patients notifying them of DSMB recommendations

Date of Submission to FDA or other Communication with FDA	Description
7/25/02	Fax to FDA providing draft press release re: DSMB recommendations and plans for FTC-301
7/25/02	Fax to FDA attaching new Informed Consent Form Addendum incorporating FDA recommendation to allow patients to stay on blinded regimen or switch to open-label FTC
7/25/02	Fax to FDA attaching draft press release re: unblinding of FTC-301
7/26/02	Fax from FDA providing Statistical Comments
7/29/02	E-mail communications with FDA and FDA fax re: FDA comments to draft press release
7/30/02	Pre-Submission of NDA Item 6 Human Pharmacokinetics & Bioavailability (NDA Volumes 2.1 – 2.50)
8/1/02	E-mail to FDA asking for clarification to 7/26/02 FDA fax comments re: statistical questions
8/1/02	Submitted Amendment 8, Protocol FTC-301A
8/20/02	Pre-Submission of NDA Item 4 Chemistry and Manufacturing (NDA Volumes 3.1 – 3.7)
9/3/02	Submitted remaining NDA sections, including electronic tapes for Items 11 and 12 (NDA Volumes 4.1 – 4.84)
9/10/02	Submitted New FTC Protocol FTC-115 and new investigator
9/20/02	Fax from FDA with Chemistry questions re: drug substance and drug product sites
9/24/02	Submitted New Protocol FTC-114 and investigator and revised 1572 for FTC-115
9/26/02	Response to FDA fax of 9/20/02 re: Chemistry questions
9/30/02	Fax from FDA providing pharmacology comments/requests re: impurities
10/1/02	Submitted response to FDA 9/30/02 pharmacology comments/requests
10/4/02	Submitted response to FDA request for information re: FTC-301A and FTC-303
10/11/02	E-mail correspondence with FDA providing updated name and address information as provided to FDA on 9/26/02 for API Step 1 intermediate manufacturing facility
10/11/02	E-mail correspondence with FDA re: e-submission for Items 11 and 12 to be submitted with 120-Day Safety Update and 48-Week FTC-301A Study Report
10/14/02	Teleconference with FDA requesting information for certain investigation sites
10/15/02	Submitted additional information to 9/26/02 submission responding to 9/20/02 chemistry questions (address for manufacturing facility)

Date of Submission to FDA or other Communication with FDA	Description
10/18/02	Submitted Response to 10/14/02 teleconference re: sites for FTC-301A and FTC-303
10/22/02	Call with FDA advising that FDA has no issues with the fileability of the NDA
10/31/02	Submitted to FDA New Investigator for FTC-301 and revised 1572s for FTC-203 and FTC-301
11/18/02	Letter from FDA re: standard review, filing date, and user fee goal
11/25/02	E-mail from FDA requesting copy of package insert and label for trade name review
11/26/02	Response to FDA request on 11/25/02 and also providing copy of 3/23/99 letter requesting review of trade name by Labeling and Nomenclature Committee
12/16/02	Letter to FDA providing CMC Amendment to CMC Section 4.3.5.2 (Vol. 4.7, page 164)
12/20/02	Fax from FDA providing Chemistry comments on NDA
12/27/02	Submitted Item 9 120-Day Safety Update Report and 48-Week Clinical Study Report for FTC-301A including electronic media for Items 11 and 12 (NDA Volumes 5.1 – 5.12)
2/19/03	E-mail to FDA re: new project manager and request for available data from ALIZE study
2/25/03	Letter to FDA from TPI about transfer of IND and NDA to Gilead
2/25/03	Letter to FDA from Gilead confirming transfer of IND and NDA
3/12/03	Fax from FDA Requesting microbiology information
3/13/03	Telephone call with FDA request and other issues
3/17/03	Letter to FDA re: preclinical data
3/18/03	E-mail correspondence with FDA re: tradename, microbiology information and package insert
3/18/03	Fax from FDA requesting statistical information
3/19/03	E-mail correspondence to FDA requesting clarification of statistical review comments
3/20/03	E-mail correspondence from FDA re: Statistical Review comments
3/21/03	Fax from FDA Requesting pharmacokinetics information
3/25/03	Submitted Response to FDA request for Microbiology Information of 3/12/03
3/31/03	Fax from FDA Requesting Pharmacokinetic information
4/1/03	Submitted Requested copies of posters presented at the 10th CROI, in Boston, MA, on 2/10-14/03
4/3/03	Teleconference with FDA re: clinical review of NDA

Date of Submission to FDA or other Communication with FDA	Description
4/4/03	Submitted Response to FDA Request for Pharmacokinetic Information
4/4/03	E-mail correspondence to FDA with Synopsis of Tenofovir/Emtricitabine Drug Interaction Study
4/6/03	E-mail Correspondence to FDA with Summary of Teleconference of 4/3/03
4/7/03	Submitted Response to FDA Request for Statistical Information of 3/18/03
4/7/03	Call from FDA re: Submission of Tenofovir/Emtricitabine Drug Interaction Study
4/7/03	E-mail Correspondence from FDA re: package insert
4/9/03	E-mail Correspondence with FDA re: Tradename
4/10/03	E-mail Correspondence to FDA re: Proposed CMC Amendment to NDA 21-500 for 200 mg capsules
4/14/03	Fax from FDA re: Request for Chemistry Information
4/15/03	Submitted Response to FDA Request of 4/14/03 for copy of FTC-105 Study Report for Section 6 of NDA
4/15/03	Submitted Response to FDA Request for Pharmacokinetic Information of 3/31/03
4/16/03	Call from FDA Requesting Dissolution Data on Commercial Formulation
4/16/03	Submitted Response to FDA request re: Dissolution Data
4/16/03	Submitted Revised Proposed Package Insert and Proposed Patient Information
4/17/03	E-mail Correspondence to FDA re: Plan for submission of Dissolution Data and submission of In Vitro Data
4/18/03	Submitted Response to FDA Request of 4/7/03 for Final Report for Study FTC-114
4/22/03	Call from FDA about analytical laboratory sites
4/24/03	Fax to FDA re: Response to 4/22/03 telephone inquiry regarding the addition of two analytical testing laboratory sites
4/25/03	Submitted CMC Amendment to NDA
4/28/03	Submitted Response to FDA Request for Pharmacokinetic information
4/28/03	Submitted New Proposed Tradename
4/29/03	Submitted Response to FDA request of 4/14/03 for Chemistry information
4/30/03	Call with FDA about submitting amendment
4/30/03	E-mail Correspondence to FDA re: CMC Amendment regarding an alternate API manufacturer
5/7/03	Fax from FDA re: Labeling Revisions
5/8/03	Teleconference with FDA re: CMC Amendment for alternate API manufacturer

Date of Submission to FDA or other Communication with FDA	Description
5/8/03	Submitted copy of response to FDA inspection of manufacturer
5/9/03	Submitted CMC Amendment for additional API manufacturer
5/9/03	Fax from FDA re: Request for Pharmacokinetics and Clinical information
5/15/03	Submitted Revised Proposed Package Insert
5/15/03	Fax from FDA re: Request for Chemistry information
5/15/03	Submitted Response to FDA Request of 5/9/03 for Pharmacokinetic information
5/16/03	Submitted Documentation to Support Tradename
5/16/03	Submitted Response to FDA Request of 5/9/03 for Clinical information
5/19/03	Fax from FDA re: Clinical Request
5/20/03	Submitted Response to FDA request for CMC Information
5/22/03	Fax from FDA re: CMC information
5/22/03	Teleconference with FDA
5/23/03	Submitted Response to FDA request for CMC Information of 5/22/03
5/28/03	Fax from FDA re: Labeling Revisions
5/29/03	Fax Correspondence from FDA re: Request for Pharmacokinetics/Pharmacometrics information
6/2/03	Submitted Response to FDA Request for Clinical information of 5/19/03
6/2/03	Fax Correspondence from FDA re: Request for chemistry information
6/3/03	Submitted Revised Proposed Package Insert
6/3/03	Call with FDA about status
6/6/03	Submitted Response to the 6/02/03 request for CMC Information
6/8/03	E-mail Correspondence to FDA re: Patient Information and Package Insert
6/10/03	Submitted Response to FDA Request of 5/28/03 for PK/Pharmacometrics information
6/12/03	Fax from FDA re: Request for Clinical Pharmacology Information
6/12/03	Call with FDA about status
6/13/03	Fax correspondence from FDA re: Labeling Revisions
6/13/03	Fax correspondence from FDA re: Postmarketing Commitments
6/18/03	Submitted Revised Proposed Package Insert and Patient Product Information
6/19/03	Submitted Response to FDA Request of 6/12/03 for Clinical Pharmacology information
6/20/03	Submitted Response to FDA Communication of 6/13/03, including Post-Marketing commitments
6/20/03	Submitted Response to CMC question on Release Testing to Detect Purity of Emtricitabine Drug Substance
6/24/03	Communications with FDA about request for Study Report
6/25/03	Submitted Response to request for Revised Specification

Date of Submission to FDA or other Communication with FDA	Description
6/26/03	Submitted Response to FDA Telephone request of 6/24/03 for Final Study Report TPI 15396
6/26/03	E-mail Correspondence with FDA re: Labeling Revisions
6/27/03	E-mail Correspondence from FDA re: Labeling Revisions and Tradename Approval
6/29/03	E-mail Correspondence to FDA Responding to FDA Labeling Comments and Submitting Revised Labeling
6/30/03	E-mail Correspondence from FDA re: Labeling Revisions
6/30/03	Call with FDA re: Labeling
7/1/03	Call with FDA re: Labeling
7/1/03	Submitted Final Package Insert and Final Patient Package Insert
7/2/03	Approval letter from FDA

GILEAD
Advancing Therapeutics.
Improving Lives.

Intellectual Property

August 29, 2003

Mary L. Severson, Ph.D., JD
Office of Technology Transfer
Emory University
1784 North Decatur Road
Suite 130
Atlanta, Georgia 30322

Re: Application for Patent Term Extension for U. S. Patent No. 5,914,331

Dear Mary:

This letter authorizes Emory University to rely upon the regulatory submissions of Gilead Sciences, Inc. and Triangle Pharmaceuticals, Inc. in support of the subject application for patent term extension.

Very truly yours,

William Schmonsees, Ph.D., JD
Sr. Director – Intellectual Property
Gilead Sciences, Inc.

sts

Gilead Sciences, Inc. 333 Lakeside Drive Foster City, CA 94404 USA
phone 650 574 3000 *facsimile* 650 522 5575

www.gilead.com